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RECRUITMENT OF *STROMBUS* VELIGERS TO THE FLORIDA KEYS REEF TRACT: RELATION TO HYDROGRAPHIC EVENTS

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ABSTRACT Recruitment of the veliger larvae of two strombid gastropods was investigated during the reproductive season (May to September) at two stations in the Looe Key National Marine Sanctuary, Florida Keys, in 1992 and 1994. The adult population of *Strombus gigas* Linné (queen conch) has been severely depleted by fishing, and despite protection of the species in Florida since 1985, there has been no recovery. *Strombus costatus* Gmelin (milk conch) is a closely related but an unexploited gastropod in Florida waters. Although the two species have very similar reproductive and larval life histories, frequent sampling showed that larval recruitment patterns were different. Early-stage veligers of *S. costatus* were very abundant, and the number of veligers decreased rapidly with size, in a typical survivorship pattern. For *S. gigas*, early- and late-stage veligers were collected in approximately equal numbers, and mid-size larvae were rare. Competent, late-stage veligers of *S. gigas* arrived in the vicinity of nursery grounds in association with thermal stratification and eastward current, indicating the nearshore presence of the Florida Current. In contrast, late-stage veligers of *S. costatus* were most often present in association with westerly flow, during periods when the Florida Current was well offshore, and the recruitment source appears to be local. These observations suggest that populations of *S. gigas* may now depend primarily on larval sources upstream from the Florida Keys in the western Caribbean Sea. Infrequent and irregular supply of larvae to the nurseries may explain the lack of population recovery for *S. gigas* in the Florida Keys.

KEY WORDS: Florida, larval supply, oceanography, recruitment, *Strombus gigas*, *Strombus costatus*

INTRODUCTION

The supply of larvae to potential juvenile habitats is well known to be an important variable in the population dynamics of benthic marine invertebrates and fishes (e.g., Yoshioka 1982, Wetley 1984, Gaines et al. 1985, Lipcius et al. 1990, Milicich et al. 1992, Peterson and Summerson 1992, Doherty and Fowler 1994, Stoner et al. 1996). Given the significance of larval supply in determining the distribution, abundance, and year-class strength of many economically significant marine species, it is imperative for effective resource management that larval sources be identified. An understanding of the physical and biological processes that mediate delivery of larvae to the subject populations is also critical.

The queen conch (*Strombus gigas* Linné) is a large, economically significant gastropod that inhabits Bermuda and southern Florida through the greater Caribbean region to Venezuela (Randall 1964). In Florida, the species was reduced to such an extent that all fishing was banned in 1985. Since that time, the population has shown no sign of recovery (Glazer and Berg 1994, Glazer and Anderson unpubl. data) and the fishing moratorium remains in effect. Larvae of other invertebrates, including certain species of lobsters and shrimp spawned in the Florida Keys, may be retained in mesoscale gyres and returned to local nurseries (Yeung and McGowan 1991, Lee et al. 1992, Lee et al. 1994, Criales and McGowan 1994). However, because there were so few queen conch in the local reproductive stock (5,800 adults along the 180 km of reef tract in 1992), Stoner et al. (1996) postulated that the

populations in the Keys are probably replenished with larvae spawned in Cuba and the western Caribbean Sea (Mexico and Belize) that are delivered via the Florida Current.

In contrast to the very small spawning population of *S. gigas* in the Florida Keys, adult *Strombus costatus* Gmelin are abundant in shallow coastal waters both north and south of the island chain. The co-occurrence of two closely related *Strombus* species in the Florida Keys and western Caribbean presents the opportunity to compare recruitment processes in the heavily exploited queen conch (*S. gigas*) and the milk conch (*S. costatus*), which is unexploited in Florida. Larvae of the two species are similar in size and general appearance, live primarily in the upper water column, and have variable but relatively similar developmental periods of 16–35 days (Davis et al. 1993). Differences in larval size-frequency between the two species and temporal differences in their delivery to Looe Key National Marine Sanctuary, combined with physical oceanographic data for the site, provide important new insights into larval sources and recruitment processes in the Keys population.

METHODS

Study Site

The Florida Keys are a chain of islands running south and west from the southern tip of the Florida peninsula to Key West. The reef tract, a series of shallow coral reefs, lies 5–10 km offshore from the islands and runs parallel to the Keys (Jaap 1984). Sampling was conducted in the lower Florida Keys near the coral reef in Looe Key National Marine Sanctuary. This particular reef is 0.8 km long, nearly emergent at low tide (range = ~1 m), oriented east-west, and located ~10 km south of Big Pine Key (Fig. 1). South of the reef, depth increases rapidly to 20 m. Areas of coral

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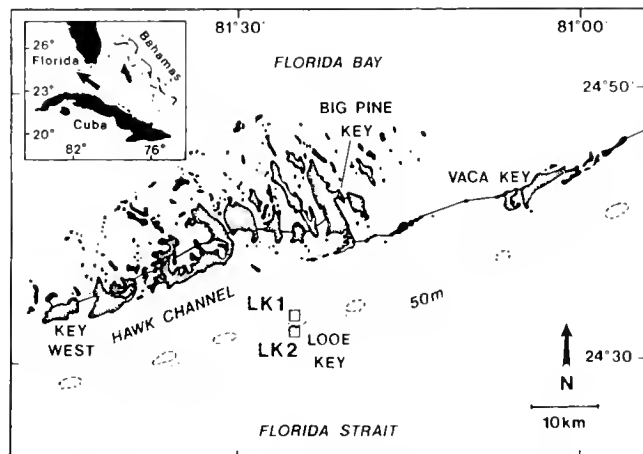


Figure 1. Map showing the lower Florida Keys and the locations of the two veliger-sampling sites near Looe Key reef.

rubble extend north from both ends of the reef, separated by a shallow (1- to 2-m-deep) bed of seagrass (*Thalassia testudinum*). Juvenile queen conch occupy these shallow rubble and seagrass areas. Adults are present in the deeper (3- to 20-m) sand and rubble habitats, where spawning occurs (Stoner et al. 1996).

Plankton samples were collected at LK1 and LK2, two sites previously studied by Stoner et al. (1996) (Fig. 1). LK1 was located in shallow (~1.5-m) water north of the reef in sand, coral rubble, and seagrass habitat. LK2 was located ~0.5 km offshore from the reef in deeper water (20–30 m), where the substrate was sand, macroalgae, and sponges.

Biological Collections

Plankton samples were collected on 33 dates at LK1 and 35 dates at LK2 (two replicates at each site) between late May and late September in 1992 and 1994. At each station, conical nets (0.5 m in diameter, 2.5 m long, 202- μ m mesh size) were towed (15 min at ~1.0 m s⁻¹) near the water surface during daylight hours. Samples were collected at the surface because *S. gigas* veligers are known to be photopositive (Barile et al. 1994) and are most abundant near the surface when conditions are relatively smooth (Stoner and Davis 1997). Tow volume was calculated from a calibrated General Oceanics flowmeter attached in the mouth of the net. Plankton samples were preserved in a buffered 5% formalin-seawater mixture.

In the laboratory, plankton samples were sorted in their entirety for veligers of *S. gigas* and *S. costatus* with a dissecting microscope (20 \times). Veligers were identified to species using the descriptions of Davis et al. (1993), counted, measured for maximum shell length (SL), and divided into three size classes: early-stage (<500 μ m SL), mid-size (500–900 μ m SL), and late-stage (>900 μ m SL) veligers. Abundance was calculated as veligers per unit volume of water sampled (veligers/10 m³) for the three size classes and for total number. Size-specific density data are useful tools for interpreting larval production and understanding transport processes. For example, early-stage *S. gigas* veligers are only a few days old (Davis et al. 1993) and reflect local larval production, whereas veligers >900 μ m represent conch ready to recruit to the benthos, are 3–4 wk old, and may have originated from a distant reproductive population (Stoner et al. 1996).

Physical Measurements

On each sampling date, data were recorded on wave height and direction, wind speed and direction, water clarity, and surface-water temperature. Water temperature and current direction and speed were measured with three General Oceanics winged current meters moored at the 30-m isobath off of Looe Key reef (24°32.5'N, 81°24.1'W) at depths of 7, 17, and 27 m. Data were filtered with a 3- and 40-h low-pass Lancos filter and subsampled at 1- and 6-h intervals, respectively. Current components were rotated into a local isobath coordinate system with +v toward 73° (i.e., alongshore in a generally eastward direction) and +u toward 163° (i.e., offshore).

RESULTS

Veliger Abundance and Length-Frequency

In 1992, mean densities of *S. gigas* were 0.60 veligers/10 m³ at the shallow site LK1 and 0.09 veligers/10 m³ at the offshore site LK2 (Table 1). Few *S. costatus* were collected in 1992. Mean density was 0.04 veligers/10 m³ at LK2, and none were collected at LK1. In 1994, densities of *S. gigas* were 0.13 veligers/10 m³ at both LK1 and LK2, and *S. costatus* had mean densities of >15 veligers/10 m³ at both stations (Table 1). However, presence was sporadic for both species. Densities of *S. gigas* were high (>1 veliger/10 m³) on only 9% (3 of 33 dates) at LK1, and only 3% (1 of 35 dates) at LK2. High densities of *S. costatus* veligers were found in 30% of the collections at LK1 and 34% at LK2, but the two species never occurred in high density at the same time.

TABLE 1.

Counts and densities of veligers of *Strombus* spp. (all stages) collected at two stations in the Florida Keys, May through September, 1992 and 1994.

Station	1992				1994			
	<i>S. gigas</i>		<i>S. costatus</i>		<i>S. gigas</i>		<i>S. costatus</i>	
	No. of Veligers	Density (no. · 10 m ⁻³)	No. of Veligers	Density (no. · 10 m ⁻³)	No. of Veligers	Density (no. · 10 m ⁻³)	No. of Veligers	Density (no. · 10 m ⁻³)
LooeKey1(LK1)	144	0.60 ± 1.14	0	0 ± 0	118	0.13 ± 0.43	15,102	16.79 ± 48.15
LooeKey2(LK2)	24	0.09 ± 0.17	13	0.04 ± 0.11	135	0.13 ± 0.30	15,533	15.88 ± 51.12
Total	168		13		253		30,635	

Density values are mean ± standard deviation. In 1994, 50 tows were made at station LK 1 and 54 tows were made at station LK 2. Sixteen tows were made at each station in 1992.

Size-frequency distributions for *S. gigas* and *S. costatus* were very different (Fig. 2). At station LK1, 89% of all *S. gigas* were early stage (<500 μm) and 10% were late stage (>900 μm). In contrast, at LK2, only 8% of the veligers of *S. gigas* were early stage and 91% were late stage. Only three mid-size (500- to 900- μm) *S. gigas* were collected. At both LK1 and LK2, over 60% of the *S. costatus* were early stage, 33–37% were mid-size, and only 0.2 (LK1) to 1.2% (LK2) were late stage (Fig. 2).

Early-stage *S. gigas* were collected sporadically and on just a few dates, primarily at station LK1 (Fig. 3). Highest densities occurred on June 13, 1992 (3.1 veligers/10 m^3), July 18, 1992 (1.5 veligers/10 m^3), and August 24, 1994 (2.0 veligers/10 m^3). Early stages were collected only twice at LK2, on June 1, 1992 (0.2 veligers/10 m^3), and August 29, 1994 (0.14 veligers/10 m^3) (not shown). The spatial pattern for the late-stage veligers of *S. gigas* was opposite that for the early stages. Late stages were very rare at station LK1, except on September 6, 1994 (0.7 veligers/10 m^3), concurrent with a similar density at LK2 in 1994. Late-stage *S. gigas* were collected sporadically at LK2 (Fig. 3), with the highest densities on August 19, 1992 (0.47 veligers/10 m^3), June 9, 1994 (0.85 veligers/10 m^3), September 1, 1994 (1.2 veligers/10 m^3), and September 6, 1994 (0.75 veligers/10 m^3).

In 1992, no *S. costatus* were collected at LK1, and only 13 individuals (0.04 veligers/10 m^3) were collected at LK2, all on July 18. High densities of early-stage veligers were collected in 1994 at station LK1. Maxima occurred on June 20 (140 veligers/10 m^3) and June 23 (58 veligers/10 m^3), with other sporadic occurrences (Fig. 4). Late stages of *S. costatus* were rare at LK1 but present in 12 of the 27 collections made at LK2 in 1994. The

densities were generally <1.0 veliger/10 m^3 , except on July 26, 1994 (8.1 veligers/10 m^3) (Fig. 4).

Relationships Between Veliger Abundance and Hydrography

The most prominent feature of currents off of Looe Key reef was the regular reversal in alongshore flow, with velocities occasionally exceeding 50 cm/sec at 7-m depth (Fig. 5). Cross-shelf currents were typically <5 cm/sec and were not particularly useful in this analysis. Strong easterly flow (+v) and increased vertical stratification in water temperature characterized periods when the Florida Current was close to Looe Key. Periods with westerly flow (-v) and low thermal stratification indicated that the Florida Current front was offshore, beyond the 30-m isobath. There were strong indications of the Florida Current at Looe Key in early July, mid- to late-August, and in mid-September 1992. In 1994, conditions indicative of the Florida Current were observed primarily in late-May through mid-June and later in mid-August to mid-September.

Because late-stage larvae provide the best indication of conch ready to recruit to shallow-water habitats, we examined their presence in terms of the position of the Florida Current front at LK2 where late stages were most abundant. On 9 of the 13 dates when late-stage *S. gigas* were collected, flow and temperature conditions indicated that the Florida Current front was close to the reef tract. On 8 of 12 dates, late-stage *S. costatus* were collected when the Florida Current was farther offshore. Fisher's exact test indicated that there was a significant ($p = 0.036$) interaction between species and the presence of the Florida Current. All high-density (>0.4

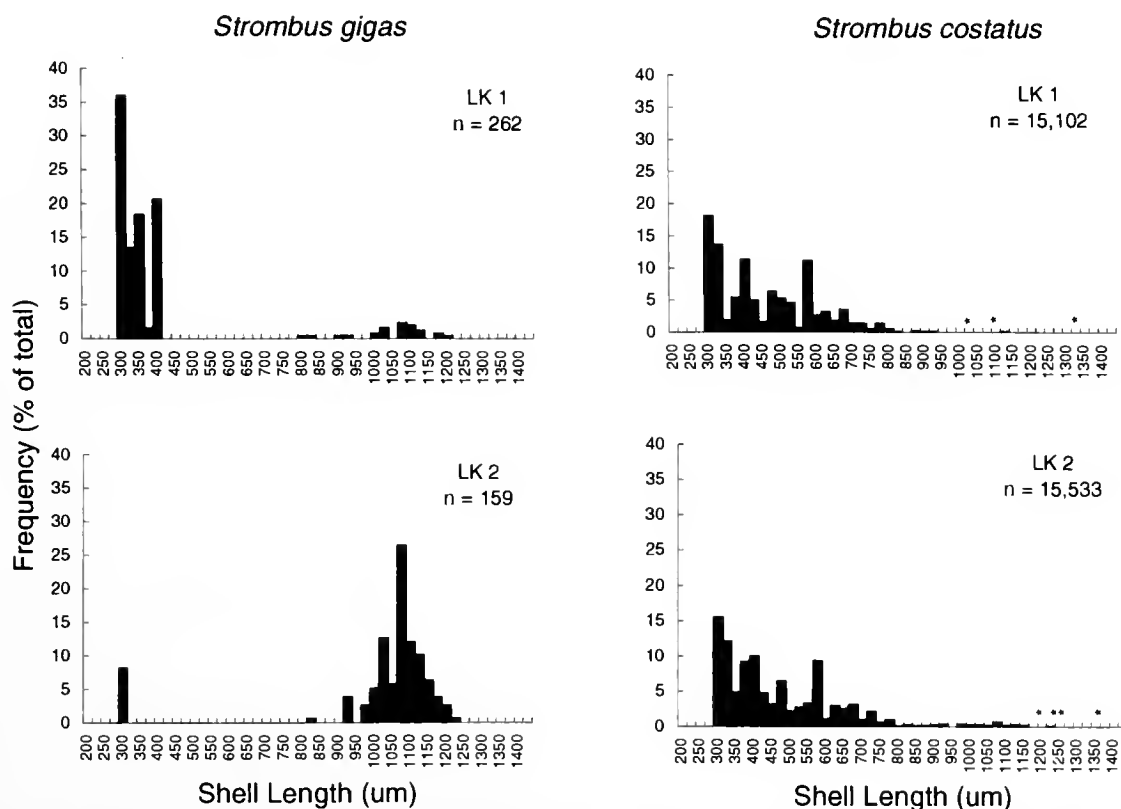


Figure 2. Size-frequency distribution for veligers of *S. gigas* and *S. costatus* at two stations in the Florida Keys. The frequency distributions for *S. gigas* represent all individuals collected in both 1992 and 1994. For *S. costatus*, only the data for 1994 are shown because only 13 early-stage veligers were collected on one date in 1992. Asterisks represent size classes comprising $\leq 0.01\%$.

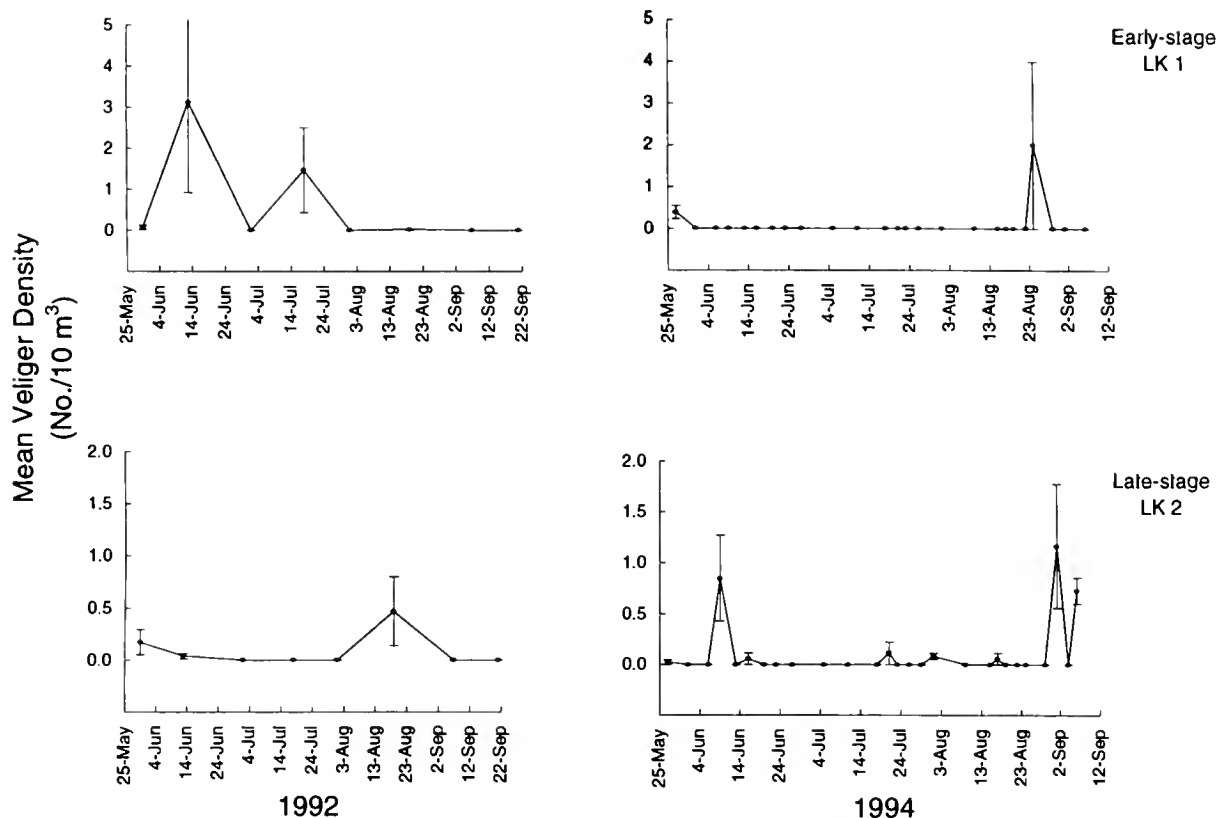
Strombus gigas

Figure 3. Density of early-stage veligers ($<500 \mu\text{m SL}$) of *S. gigas* at station LK1 and late-stage veligers ($\leq 900 \mu\text{m SL}$) at station LK2 in 1992 and 1994. Values shown are mean \pm standard error ($n = 2$). Note that the scales are different for early- and late-stage larvae.

veligers/ 10 m^3) occurrences of late-stage *S. gigas* (August 19, 1992, and June 9, September 1, and September 6, 1994) were associated with distinct easterly flow and vertical stratification of the water column (Figs. 3 and 5). The highest densities of late-stage *S. costatus* (July 18, 1992, and June 27, July 26, and August 1, 1994) always occurred when currents were westerly and there was little stratification (Figs. 4 and 5). Late-stage *S. costatus* were present on one occasion at LK2, during a period of high easterly flow (June 9, 1994), but the density was only $0.36 \text{ veligers}/10 \text{ m}^3$.

DISCUSSION

Mesoscale gyres can affect the retention and recruitment of larval fish and invertebrates in the nearshore environment of the lower Florida Keys (Lee et al. 1992, Lee et al. 1994). Evidence exists for the retention of penaeid shrimps on the Tortugas and Pourtales Gyres (Criales and McGowan 1994, Criales and Lee 1995), and similar mechanisms may be responsible for retaining the larvae of certain scyllarid lobsters in the vicinity of the Florida Keys shelf (Yeung and McGowan 1991). With a 2- to 4-wk-long larval period for *Strombus* spp. (Davis et al. 1993), similar to that of these shrimps and lobsters, it is plausible that the retention and recruitment of *Strombus* spp. would be affected by mesoscale gyres in the Straits of Florida. However, two strong lines of evidence suggest that this was not the case for larvae of *Strombus* spp. in the two years surveyed.

First, it is very unlikely that most of the veligers of *S. gigas* were produced by spawners in the Florida Keys. Estimates for the

total number of adult queen conch in the entire Keys region was just 5,800 individuals in 1992 and 9,200 in 1994 (Glazer and Anderson unpubl. data). There were relatively few newly hatched veligers of *S. gigas* at Looe Key, despite the fact that a large proportion of the total Keys reproductive stock (10% in 1992 and 20% in 1994) occurred in the well-patrolled environment of Looe Key National Marine Sanctuary. It is very unlikely that the densities of late-stage *S. gigas*, often exceeding the densities of early stages collected at numerous locations surveyed in the Florida Keys between 1992 and 1994 (Stoner et al. 1996, this study), could be derived from these low concentrations of early stages, particularly when mid-sized larvae are extremely rare.

Second, there are large spawning stocks of *S. gigas* in Mexico and Belize, and recruitment of late-stage veligers to Looe Key during periods of high eastward flow is consistent with the hypothesis that they have a source in the western Caribbean Sea. The plausibility of this source of larvae and the associated transport mechanism is supported by examination of the larval development period in combination with what is known about near-surface circulation between the Yucatan Strait and Florida Keys. Trajectories of satellite-tracked drifters show that surface water passing through the Yucatan Strait and into the Loop Current of the eastern Gulf of Mexico can reach the Florida Keys area in 30–35 days (Kinder 1983). When the Loop Current is less well developed and surface flow is more direct from the Caribbean coast of Mexico to Florida (approx. 700 km), the transit time could be as short as 10 days, with a current velocity of 0.8 m/sec , observed by Kinder

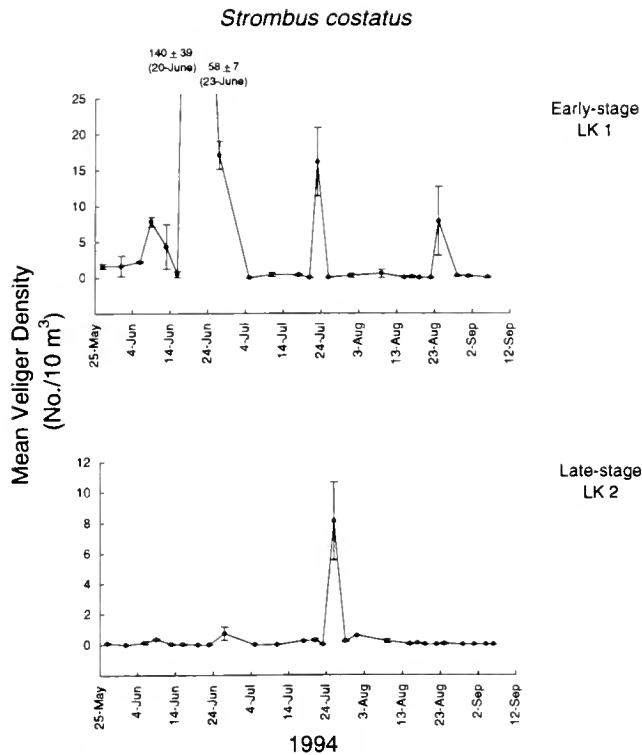


Figure 4. Density of early-stage veligers (<500 μm SL) of *S. costatus* at station LK1 and late-stage veligers (≥ 900 μm SL) at station LK2 in 1994. Values shown are mean \pm standard error ($n = 2$). Numbers collected in 1992 were too low to plot. Note that the scales are different for early- and late-stage larvae.

(1983). Consequently, the larval period of 2–4 wk in *S. gigas* (Davis et al. 1993) is sufficiently long for transport from large reproductive populations in Mexico, Belize, and Honduras. The scarcity of early-stage veligers of *S. gigas* in the Florida Current south of Looe Key precludes the north shore of Cuba as a source because drifter model trajectory estimates of arrival times are on the order of 4–6 days, if at all (Chen 1996). Concentrations of late-stage *S. gigas* are known to be high in the Florida Current 35 km south of the middle Keys (Stoner et al. 1996), and the arrival of *S. gigas* in association with easterly flow at Looe Key indicates that larvae of Caribbean origin are being delivered by the Florida Current. Although genetic studies have shown a high degree of similarity among populations of *S. gigas* in the Caribbean and Florida (Mitton et al. 1989, Campton et al. 1992), this study provides the first oceanographic data indicating that the Florida Keys population of queen conch is now at least partially dependent on upstream sources.

S. costatus has a larval life history (Davis et al. 1993) and vertical migration behavior (Stoner and Davis 1997) similar to that of *S. gigas*, and it could be expected that some veligers of *S. costatus* recruit from reproductive populations in the Yucatan. Nevertheless, recruitment of late-stage *S. costatus* larvae was associated with Florida Current conditions on only one occasion. Recruitment of *S. costatus* in the Keys may be influenced by mechanisms similar to those affecting alpheid shrimps. Ciales and McGowan (1994) found that alpheid larvae were abundant only in nearshore waters in the Florida Keys, and they proposed that the entire larval development occurred in coastal waters, unaffected by the Florida Current or mesoscale gyres. The size-frequency distri-

bution for veligers of *S. costatus* suggests a typical survivorship pattern, with a high abundance of early stages and relatively rare late stages. Also, *S. costatus* occurred at Looe Key primarily when temperature and current conditions indicated that the Florida Current front was well offshore. This may explain the low numbers of larvae collected at Looe Key in 1992. Only one collection was made during a period of strong westerly flow in 1992, on July 18, when all of the larvae were collected. Periods of low thermal stratification were rare in 1992. However, in 1994, there were long periods of westerly flow and low stratification, and the vast majority of *S. costatus* was collected during these periods (Fig. 5).

The inshore distribution and spawning of *S. costatus* may explain the association of larvae with westerly current and the shelf water mass. Although populations of *S. costatus* have not been quantified, the species is very abundant near the islands and in Florida Bay and is relatively rare near the reef tract (pers. observ.). Consequently, the larvae are generally outside the influence of the Florida Current, and the most important source of recruitment for *S. costatus* is probably local.

Our findings have important management implications. It is

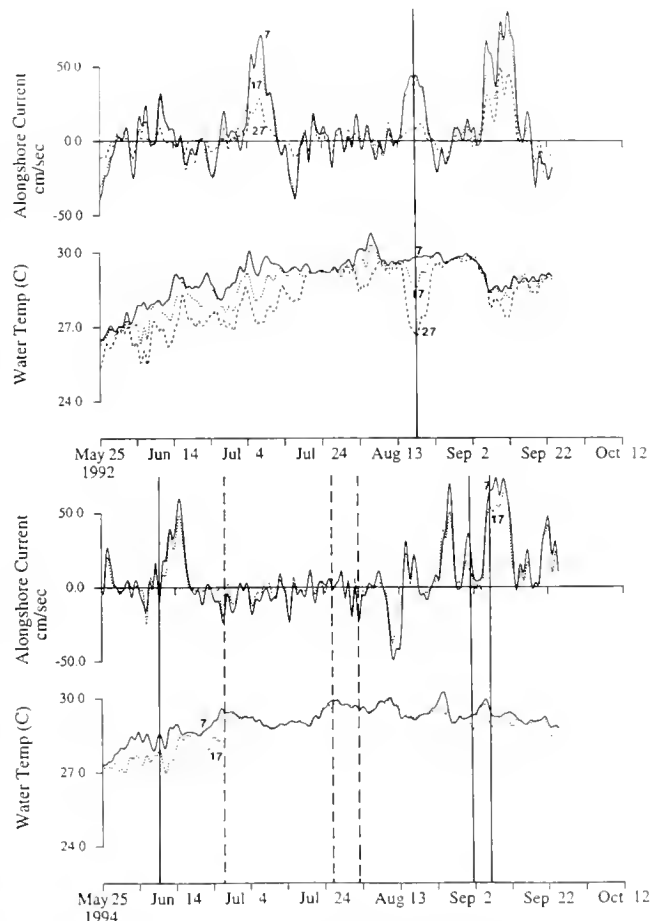


Figure 5. Alongshore current and water temperatures (Temp) off-shore from Looe Key reef, May through September, 1992 and 1994. Solid-line plots represent readings at 7 m, dotted lines are readings at 17 m, and dashed lines are readings at 27 m in 1992. In 1994, the meter at 27-m depth failed to record. Solid vertical lines indicate the sampling dates when high densities (>0.4 veligers/10 m³) of late-stage *S. gigas* were collected. Dashed vertical lines show the dates when high densities of late-stage *S. costatus* were collected.

very likely that the large populations of *S. gigas* once known in the Florida Keys were sustained by local spawners, as are populations of *S. costatus*. Today, however, with severe overfishing, the only reproductive stocks of *S. gigas* lie several kilometers offshore, along the reef tract (Stoner et al. 1996). The larvae produced at the outer shelf are easily lost to the Straits of Florida, the densities of late-stage larvae in the coastal water mass are practically zero, and the Florida Current is now the primary recruitment source for *S. gigas*. Recruitment to juvenile populations in *S. gigas* is known to be dependent on larval supply (Stoner et al. 1996), but deliveries of settlement-stage larvae by meanders of the Florida Current are probably too infrequent and irregular to sustain or rebuild a large spawning population in the Florida Keys. For example, Florida Current conditions did not occur at Looe Key between mid-June and mid-August 1994, and almost no late-stage *S. gigas* were collected during that period. Lack of recruitment during the warmest part of the summer season is likely to have a significant negative effect on year-class strength for 1994, particularly at this northern extreme of the species' geographic range.

Rehabilitation of queen conch stocks in the Florida Keys may now depend on transplants of spawners or the release of hatchery-reared juveniles. Unfortunately, stock enhancement through juve-

nile release is difficult and expensive and has a history of low success (Stoner 1994, Stoner and Glazer in press). Wise management and transgenerational enhancement of marine fishery resources will depend on extensive knowledge of recruitment processes and metapopulation dynamics.

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ABUNDANCE AND DISTRIBUTION OF QUEEN CONCH VELIGERS (*STROMBUS GIGAS* LINNE) IN THE CENTRAL BAHAMAS. I. HORIZONTAL PATTERNS IN RELATION TO REPRODUCTIVE AND NURSERY GROUNDS

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ABSTRACT Veliger larvae of the large gastropod *Strombus gigas* (queen conch) were collected over a 7-y period at a reproductive site in the Exuma Sound, in adjacent tidal inlets, and on the Great Bahama Bank near Lee Stocking Island, Exuma Cays, Bahamas. Although the spawning season for queen conch in the region occurs from April through October, larval abundance was highest during mid summer, between late June and August, when temperatures were $>28^{\circ}\text{C}$. Metamorphically competent larvae were most abundant in July and August and made up a small percentage of the total count, reflecting high natural mortality. Although there was considerable interannual variation, veliger densities near Lee Stocking Island (typically 1–2 individuals/10 m³) were much higher than estimates made in the eastern Caribbean and Florida Keys. Because most of the collected larvae were newly hatched, regional differences in larval abundance appear to be associated with size of local spawning stock. Highest densities of larvae were found on the Great Bahama Bank directly associated with axes of tidal currents and decreased with distance onto the bank. Intensive sampling in one tidal flow field showed that total larval densities as well as densities of late-stage veligers were highest at a well-established nursery site. Larval transport and retention may explain the general occurrence of nurseries at locations where water from the Exuma Sound flows onto the Great Bahama Bank on flood tides. Large, stable aggregations of juvenile queen conch were consistently supplied with high densities of larvae and were directly associated with tidal pathways. In contrast, more ephemeral aggregations were characterized by low or inconsistent veliger densities (particularly late-stage larvae) and were generally outside primary tidal current pathways. Queen conch distribution appears to be directly related to the horizontal supply of larvae.

KEY WORDS: Bahamas, larval transport, oceanography, recruitment, *Strombus gigas*

INTRODUCTION

The large gastropod *Strombus gigas* Linne (queen conch) is an important fisheries resource in the wider Caribbean region (Berg and Olsen 1989, Appeldoorn 1994). Despite its culture in hatcheries for nearly 20 y (Creswell 1994, Davis 1994a), knowledge of the natural history and field distribution of the veliger larva is limited to the most basic facts. The spawning season lasts 24–36 wk and varies slightly throughout the Caribbean, depending on temperature and photoperiod (Stoner et al. 1992). After 3–5 days, conch veligers hatch from their benthic egg masses at 300 μm shell length (SL) (D'Asaro 1965, Davis 1994a); they then live in the water column for 18–26 days, depending on physical and trophic conditions (Laughlin and Weil 1983, Siddall 1983, Davis et al. 1993). Laboratory experiments showed that queen conch larvae are positively phototactic and negatively geotactic, suggesting that most will be found near the sea surface (Barile et al. 1994). At 0.9–1.0 mm SL, the veligers are competent to undergo metamorphosis on contact with a variety of substrata found in natural nursery areas (Davis 1994b, Davis and Stoner 1994).

The morphological development of queen conch veligers was first described by D'Asaro (1965), but a comparative description of larval morphology adequate to identify different Caribbean *Strombus* species has only recently become available (Davis et al. 1993). The only published data on larval abundance in the field are from Lee Stocking Island (LSI), in the central Bahamas (Chaplin 1989, Chaplin and Sandt 1992, Stoner et al. 1992, Stoner et al.

1994), and from a one-time survey of 14 stations in the eastern Caribbean Sea (Posada and Appeldoorn 1994).

With the rapid decline in queen conch populations throughout the species' geographic range (Appeldoorn et al. 1987, Appeldoorn 1994), it is increasingly important to understand larval transport and recruitment processes on the local and regional scale. Berg and Olsen (1989) pointed out the possibility that the conch fishery in many nations might depend on upstream sources of larvae and that this should be taken into account for effective management of the species. New data, for example, suggest that recovery of the severely depleted Florida conch population will depend on the transport of larvae from Caribbean nations (Stoner et al. 1996a, Stoner et al. 1997).

Here, we summarize the findings from 7 y (1988–1994) of field sampling for queen conch veligers near LSI. Our primary purpose is to provide seasonal data on the abundance of conch larvae and examine distribution over a range of habitats, from spawning sites on the island shelf to shallow regions on the Great Bahama Bank near historically important nursery grounds. We also compare annual abundance and size structure of veligers collected at some nursery areas that contain large and stable juvenile populations and at some that have been ephemeral. These data are useful in interpreting the relationships between larval supply and juvenile population size and help to explain patterns of nursery distribution.

METHODS

Study Sites

During seven spawning seasons (1988–1994), plankton collections were made in the vicinity of LSI, Bahamas ($23^{\circ}46'\text{N}$, $76^{\circ}06'\text{W}$) (Fig. 1), where there is only light fishing pressure. Large populations of queen conch juveniles occur on the Great Bahama

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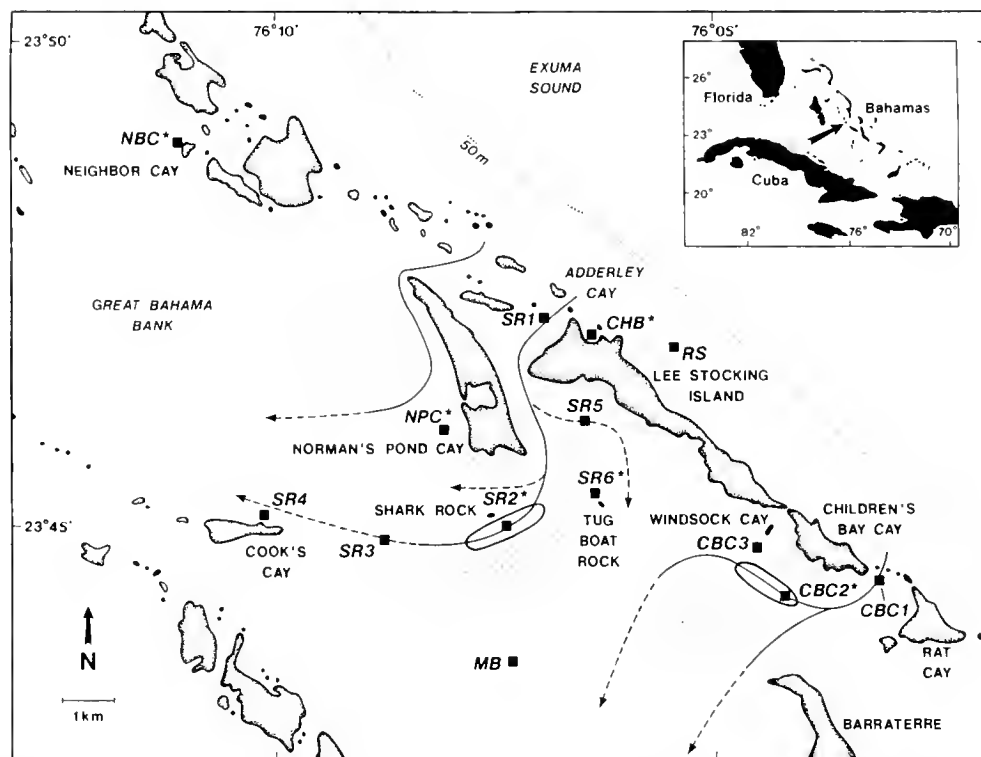


Figure 1. Map showing 13 plankton collection stations in the vicinity of LSI, Exuma Cays, Bahamas. Stations were located in and around known nursery grounds. Station labels with asterisks indicate sites that were occupied by juvenile conch. Solid lines and arrows indicate the primary pathways for flood tidal currents. Dashed lines show secondary pathways. Elliptical areas represent the general locations of long-term nurseries near Shark Rock (SR2*) and Children's Bay Cay (CBC2*). Stippled areas represent shallow sand bars.

Bank (Stoner et al. 1994), and adults are abundant in the Exuma Sound (Stoner and Schwarte 1994, Stoner and Ray 1996). The islands of the Exuma chain are bordered on the west by shallow banks (mean depth, ~4 m) and on the east by the deep Exuma Sound. On flood tides, oceanic waters from the Exuma Sound flow onto the bank through numerous inlets on the flood tide and mix with bank water. For the purpose of this study, we assumed that queen conch larvae were carried from the offshore spawning sites to the bank on the tidal currents. Velocities through the 5- to 8-m-deep inlets typically reach 50–100 cm/sec at maximum flood. The tide is semidiurnal with a range of approximately 1 m. Winds are predominately from the ESE during the summer spawning season, with wind speeds typically 3–6 m/sec (Caribbean Marine Research Center, unpubl. data).

To observe seasonal variation in veliger density in a reproductive area, plankton collections were made at station RS, located approximately 1 km east of LSI on the island shelf in the Exuma Sound (Fig. 1). Adult conch are abundant at RS on an 18-m-deep platform covered with sand and algae (Stoner and Sandt 1992). Sampling schedules are explained under *Plankton Collections*.

Drifter studies have shown that tidal waters from the Exuma Sound flood through inlets north and south of LSI and into two corresponding flow fields. The north tidal system passes over conch nurseries near Shark Rock and Tugboat Rock, and the south system passes over a nursery west of Children's Bay Cay (Stoner et al. 1994, Stoner et al. 1996b) (Fig. 1). Plankton collections were made to determine seasonal and geographic distribution of veligers with respect to the tidal current patterns. Plankton collections were made at four stations along the primary axis of tidal flow between Adderley Cay and Cook's Cay (stations SR1, SR2*, SR3, and

SR4) and at two stations along a secondary axis of tidal flow between LSI and Tugboat Rock (SR5 and SR6*). Plankton collections were also made at three stations in the Children's Bay Cay flow field between the inlet and Windsock Cay (CBC1, CBC2*, and CBC3). One additional station was sampled in the middle of the bank (MB), between the two primary flow fields but well outside the primary tidal currents. No juvenile conch have ever been observed at the nonnursery stations, although adults are occasionally observed over most of the Great Bahama Bank and island shelf adjacent to LSI.

Stations located in known nursery grounds are indicated with asterisks in the station code (e.g., SR2*). The Shark Rock (SR2*) and Children's Bay Cay (CBC2*) nurseries have been occupied by large, stable juvenile queen conch populations that often contain between 10^4 and 10^5 individuals (Stoner et al. 1996b). Juvenile populations at SR6* usually contain $<10^4$ individuals and have been more ephemeral than those at SR2* and CBC2* (Stoner et al. 1996b). All of these nursery sites are located in shallow water (2–3 m) and are covered primarily with sparse to medium-density seagrass (*Thalassia testudinum*), a similar habitat on most of the bank.

Plankton collections were made at three other nursery sites known to be occupied by low numbers of queen conch juveniles. Station NPC* was west of Norman's Pond Cay and is located in a tidal flow field that begins at the north end of this cay (Fig. 1). Station NBC* was located just off the north beach of Neighbor Cay, which is approximately 8 km north of LSI. Both of these nurseries are occupied by a few thousand juvenile conch that appear in some years and are absent in others (Sandt and Stoner 1993, Stoner, unpubl. data). The third station was off Charlie's Beach (CHB*) on the windward side of LSI (Fig. 1). This island

shelf site is occupied by only a few hundred juvenile conch, which appear irregularly. All three sites are protected from the prevailing wind (ESE), and they are in shallow (1- to 2-m) water, where the bottom is mixed seagrass and sand.

Plankton Collections

Surface plankton collections were made by towing simple conical nets (0.5 m in diameter, 2.5 m in length) from a small boat. The nets were towed in the upper 1 m of the water column for 15–20 min at approximately 1 m/sec. The volume of water sampled, typically 200–250 m³, was calculated with a calibrated General Oceanics flowmeter suspended off-center in the mouth of the net. Unless otherwise specified, replicate tows were made at each station. A mesh size of 202 μ m was used to collect all larval stages including newly hatched larvae.

During the first year of sampling (1988), collections were made at RS, SR1, and CBC1 from March to October at 2-wk intervals to examine larval densities over the entire spawning season. Because very few larvae were collected in March, April, and October (see Results), sampling was concentrated between May and September in subsequent years at all stations. Nursery sites were sampled every 2 wk in 1989 and 1990 and on an approximately weekly schedule in 1992–1994. Only the reproductive site was sampled in 1991.

Collections were made at nursery station SR2* in 1989 to test for possible day-night variation in estimates of veliger density. Plankton tows were made at the high tide during midday and at midnight on August 2 and 17. Because daytime sampling yielded higher densities of conch larvae (see Results), all subsequent collections were made during midday.

The effects of tidal period on veliger density were examined at the inlet station, SR1, north of LSI, where tidal current velocities are in direct phase with tide height (i.e., zero velocity occurs with high and low water). Plankton samples were collected on 20 dates in 2-day pairs between July 14 and August 28, 1990, at approximately 1-wk intervals. Collections were made every hour, when possible, between low and high tide, with greatest effort concentrated on the midtide period. Sixteen to 18 collections were made between 2 and 4 h after low water. Seven sets of tows were made 1 h after low water, and five sets were collected 5 h after low water. The results of this analysis provided the rationale for sampling time at the bank sites. All collections used in seasonal and annual analysis of veliger density in the inlets (including 1988 and 1989) were made 2 h after the beginning of the flood tide. Because of the time required for Exuma Sound water to pass onto the Great Bahama Bank, collections at stations on the bank were made during the last 2 h of the flood tide. Collections at RS, in the open sound, were made independent of tide.

Larval Identification and Staging

From 1988 to 1991, plankton samples were sorted live within 4 h of collection. With the aid of a dissecting microscope (20 \times), live queen conch veligers could be positively identified by distinct orange pigment cells on the propodium and purplish-brown pigment on the edges of the velar lobes (Davis et al. 1993). Laboratory-reared veligers were used to verify distinguishing characteristics of the most similar and abundant veligers, *S. gigas* and *Strombus costatus*. From 1992 to 1994, the samples were preserved in 5% buffered formalin immediately after collection, and

sorting was accomplished within 6 mo, using shell features described by Davis et al. (1993).

Each sample was sorted by pouring multiple subsamples into gridded Petri dishes. If the volume of settled plankton was high, the sample was split once with a Folsom plankton splitter before being sorted. Conch veligers from each plankton tow were counted and measured for SL (apex to siphonal canal) with an ocular micrometer. For this study, veligers were divided into three size classes: newly hatched (300–500 μ m SL), midsize (500–900 μ m), and late stage (>900 μ m). Late-stage larvae were either competent for metamorphosis or very nearly so.

Data Analysis

Densities are reported as number of veligers/10 m³. The mean of the replicate tows was calculated, and in some analyses, means of means were used to describe density for a particular month or season. One-way analysis of variance (ANOVA) was used to compare larval densities estimated for different tidal stages at SR1 and for day-night comparisons at SR2*.

RESULTS

Temporal Variation

Monthly plankton collections made in 1988 at the reproductive site and in the tidal inlets (Fig. 2) were consistent with the known summer spawning season of *S. gigas* in the Exuma Cays. Veligers were first collected on June 2 at RS, on June 6 at SR1, and on June 20 at CBC1. Larval densities were highest (0.26–4.46 veligers/10 m³) in the surface water during midsummer (June through August), and no veligers were found after the end of September. These results provided the rationale for sampling between May and September in subsequent years.

Larval densities were 3–10 times higher during the day than at night during the peak reproductive season in 1989 at nursery site SR2* (Fig. 3). The difference was significant on both August 2 [ANOVA, $F_{(1,2)} = 16.0$, $p = 0.057$] and on August 17 [$F_{(1,2)} = 106.1$, $p = 0.009$]. All of the veligers collected were newly hatched with no difference in SL between day and night (day: mean = 436 μ m; SD = 28; $n = 49$; night: mean = 426 μ m; SD = 13; $n = 5$).

Veliger density at the reproductive site was generally low (<1.5 veligers/10 m³) and variable. High abundance (2.6–7.4 veligers/10 m³) occurred only during June 1990 and early July 1991 (Fig. 4). Most veligers were newly hatched, although midsize veligers were abundant in 1991, and late-stage veligers were present later in the spawning season during both years. The highest observed density for newly hatched veligers was 7.44 veligers/10 m³, which was 13 times the maximum for midsize larvae (0.56 veligers/10 m³) and almost 30 times higher than the maximum for late-stage larvae (0.26 veligers/10 m³). No veligers were collected at the reproductive site on several sampling dates during the spawning season in either 1990 or 1991.

In the inlet north of LSI (station SR1), where tidal variation was examined, the highest density of queen conch veligers occurred 2 h after the onset of flood tide (Fig. 5). Because of large variation in densities, tidal phase did not have a significant effect on estimates of veliger density [ANOVA, $F_{(4,58)} = 0.652$, $p = 0.628$]. Nevertheless, all subsequent sampling at SR1 was conducted at the time of maximum density (i.e., 2 h after slack low water) in the daytime.

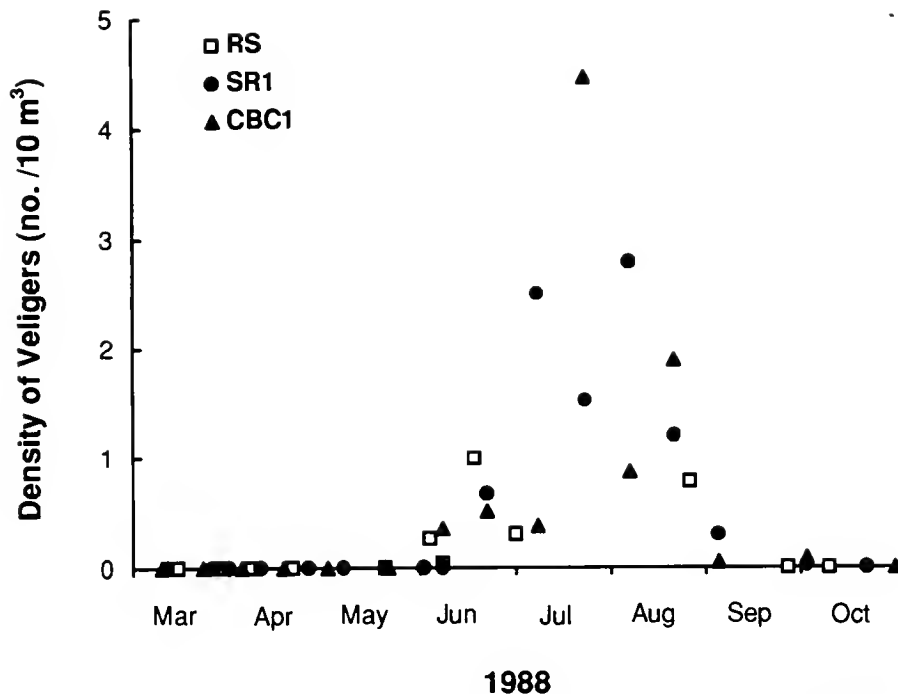


Figure 2. Density of queen conch veligers during 8 mo in 1988 at the reproductive site (RS) (12 sampling dates) and at two inlet sites (17 dates at SR1; 15 dates at CBC1). Values are means of two replicate tows.

Spatial Variation

Veliger density during three spawning seasons was consistently higher at inlet station SR1 (Fig. 6) than at RS (Fig. 4). Veligers were present in the inlet from May through September, with peak abundance in July and August. Highest veliger densities occurred in 1989 (5.5 veligers/10 m³) and 1992 (7.2 veligers/10 m³). In 1989 and 1990, veligers ranged from newly hatched to 600 μ m SL. In 1992, 95% of the veligers collected in the inlet were newly

hatched; midsize veligers were occasionally found in the inlet, with a high value of 0.24 veligers/10 m³ (Fig. 7). Late-stage veligers were collected at SR1 in June, in July, and at the end of the season in September, with maximum density at 0.26 veligers/10 m³, similar to maxima at the reproductive site (Figs. 4 and 7).

In 1988, densities of queen conch veligers in the Children's Bay Cay tidal system decreased with distance from the Exuma Sound onto the Great Bahama Bank. The mean density of veligers in July and August was four times higher in the conch nursery (station CBC2*) than at the nearby bank station CBC3, but was approximately half the density observed at inlet station CBC1 (Table 1). Veligers in this flow field ranged in size from newly hatched to 600 μ m SL, except for three late-stage veligers (1,350 μ m SL) collected at CBC2* in mid-July. The mean density of veligers at CBC1 (1.90 veligers/10 m³) was nearly identical to the density at the adjacent inlet site SR1 (1.99 veligers/10 m³) (Table 1). Relatively low veliger densities were found at station SR5, where juvenile conch have never been observed.

In 1989, veliger distribution was examined along the Shark Rock flow field, where the tidal current is confined to a more distinct channel than that near Children's Bay Cay. In July and August, mean larval densities were high (>2.3 veligers/10 m³) all along the flow field from the inlet (SR1) to Cook's Cay (SR4) (Table 1), with highest density (4.20 veligers/10 m³) at the conch nursery (SR2*). Only two larvae were collected during the entire season at the station in the middle of the bank (MB), yielding a very low mean density (0.01 veligers/10 m³). Veligers collected during 1989 were 300–600 μ m in SL, with no late-stage veligers collected at any site during this season.

Sampling in the Shark Rock flow field during 1992 yielded a trend similar to that observed in 1989 (Table 1). The highest mean density during peak reproductive season (July to August) in 1992

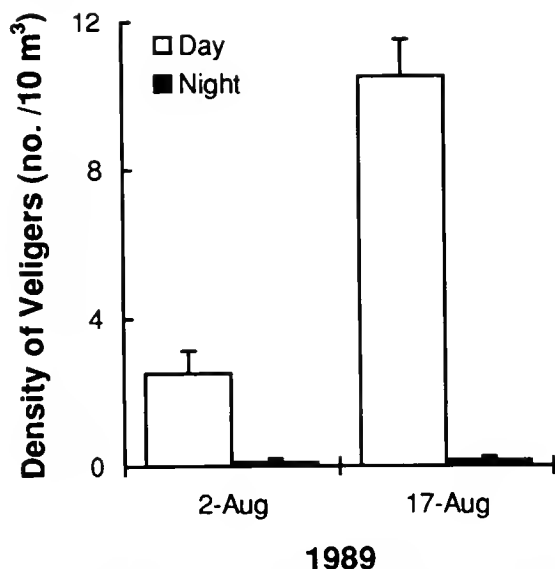


Figure 3. Day versus night comparisons for density of queen conch veligers in surface waters at station SR2* in August 1989 (mean \pm SE, n = two tows per sampling period).

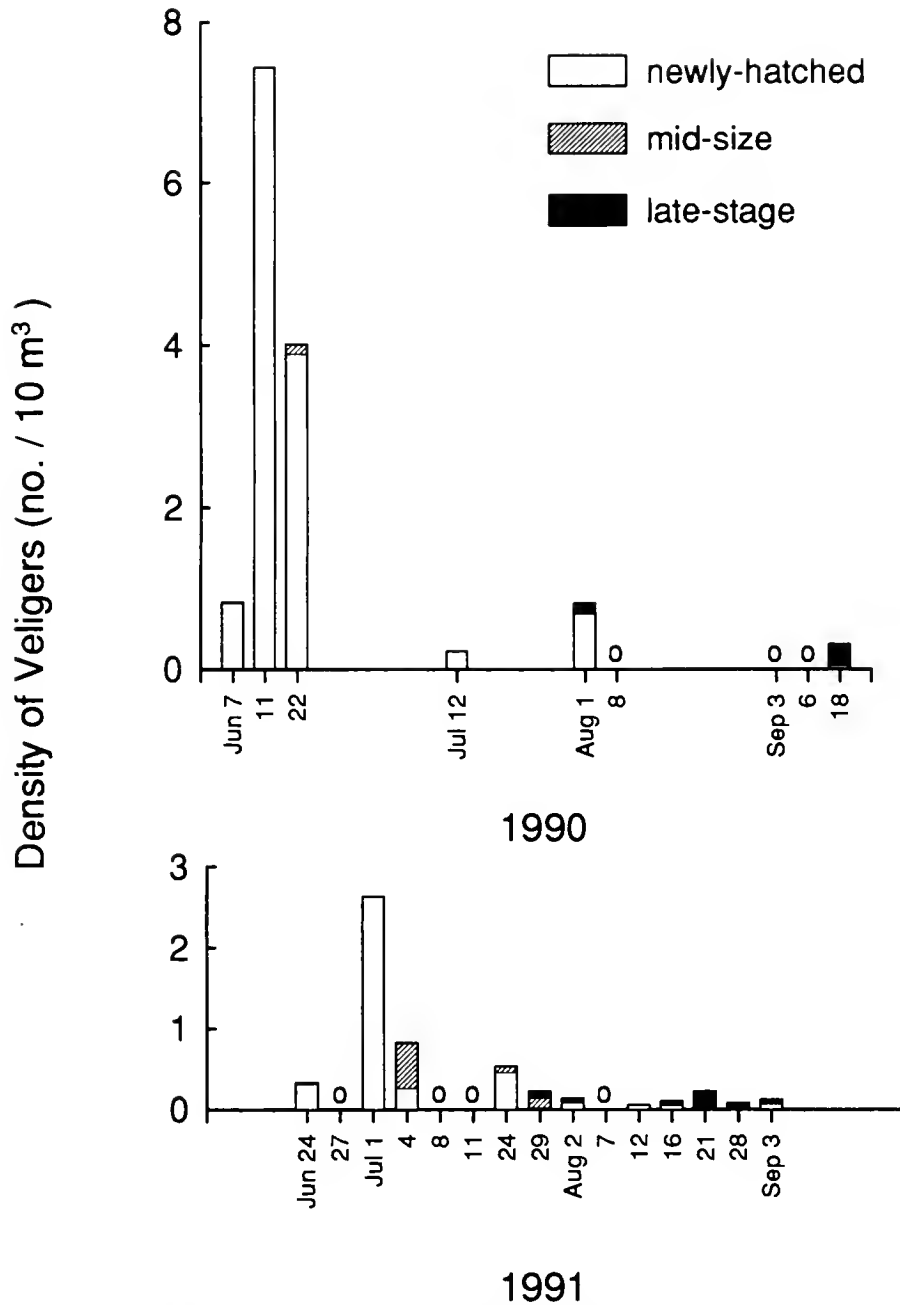


Figure 4. Mean density of queen conch veligers collected at the reproductive site (RS) during the 1990 and 1991 reproductive seasons ($n =$ two tows per sampling date). Densities are reported by size categories. Newly hatched veligers were 300–500 μm SL, midsize veligers were 500–900 μm SL, and late-stage veligers were >900 μm SL.

occurred at SR2* (3.61 veligers/ 10 m^3). The mean density at SR6* (1.98 veligers/ 10 m^3) was similar to that at the inlet site SR1 (1.94 veligers/ 10 m^3) (Table 1).

High numbers of larvae collected in the Shark Rock flow field in 1992 permitted analysis of size frequency (Fig. 7). Conch veligers were present at the station farthest from the sound (SR4), but all were newly hatched larvae. Midsize and late-stage veligers were found only in the vicinity of existing nurseries (SR2*, SR6*) and at the inlet (SR1) (Tables 2 and 3). There was an influx of midsize and late-stage veligers through the inlet (SR1) to Shark Rock (SR2*) and Tugboat Rock (SR6*) nurseries on one date, July

2, 1992 (Fig. 7). A high mean density of veligers was also found at the Children's Bay Cay nursery (CBC2*) during the peak larval season, twice the value recorded in 1989 (Table 1).

Veliger Densities in Stable Versus Ephemeral Nurseries

Annual variation in the density of queen conch veligers was observed both at the stable, long-term nursery sites (SR2* and CBC2*) and near the most ephemeral populations (SR6* and NBC*) (Table 1; Figs. 7–9). All densities increased from 1992 to 1993; however, during these years when all four nursery sites were

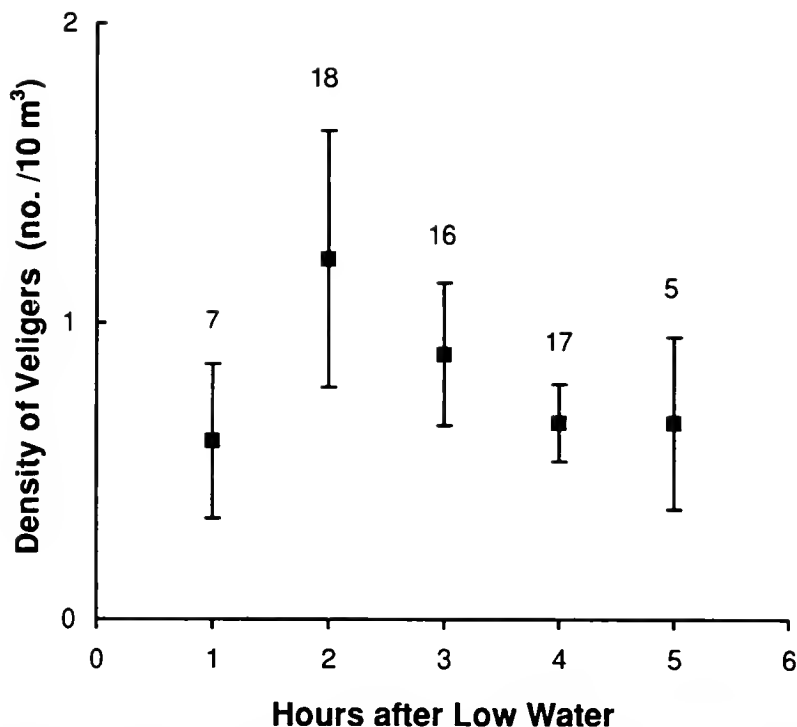


Figure 5. Density of queen conch veligers collected at inlet station SR1 during flood tides in July and August 1990 (mean \pm SE). The values above the error bars are the numbers of sampling periods. Two replicate tows were made for each period.

sampled, annual variation was less at the two stable nurseries (1.1 and 2.2 times, at CBC2* and SR2*, respectively) than at the ephemeral sites (4.0 times) (Table 1). Spatial patterns of veliger density over the Great Bahama Bank were not always consistent. For example, in 1994, veliger density was very high at SR2*, but low in the adjacent flow field at CBC2* (Table 1). Nevertheless, SR2* and CBC2* had the highest mean densities of veligers

among all sites sampled in every year and the highest densities of late-stage veligers (Tables 2 and 4).

Midsize and late-stage veligers were relatively rare at sites with ephemeral populations of queen conch. Veligers >0.5 mm SL were collected in the vicinity of NBC*; however, this occurred only during peak reproductive months (June through August) (Table 5, Fig. 9), and densities were typically an order of magnitude less

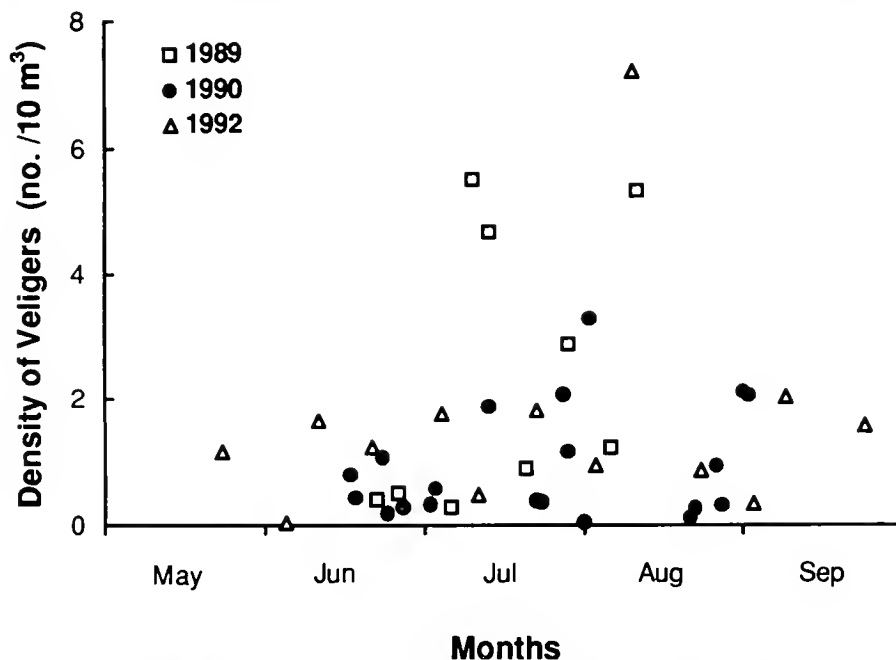


Figure 6. Density of queen conch veligers collected at inlet station SR1 during three spawning seasons (1989, 1990, and 1992). Each point represents the mean for two plankton collections.

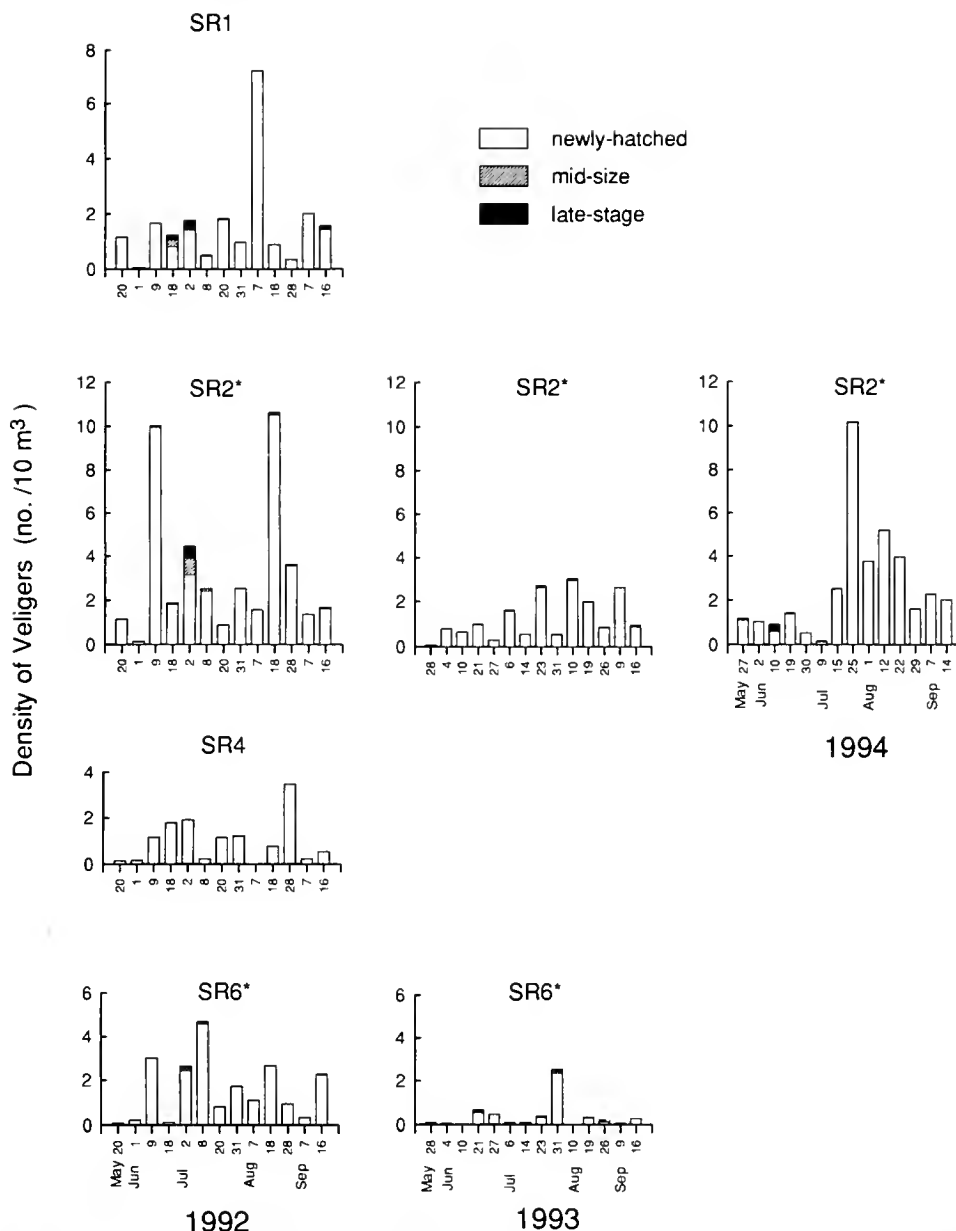


Figure 7. Mean density of queen conch veligers collected at seven stations in the Shark Rock flow field during three spawning seasons (1992, 1993, and 1994). Densities are reported by size category. Each column represents the mean for two plankton collections made on 13–14 sampling dates between May and September. Newly hatched veligers were 300–500 μm SL, midsize veligers were 500–900 μm SL, and late-stage veligers were >900 μm SL.

than those at the sites with larger juvenile populations. In the 2 y of sampling at SR6*, late-stage larvae were common (≥ 0.1 veliger/10 m³) only on single dates in both 1992 and 1993 (Table 3). The ephemeral nursery at CHB* was sampled during only one season (1992) and yielded the lowest mean density of veligers among stations sampled that year (Table 1); midsize veligers were never collected, and only two late-stage veligers were collected (Fig. 9).

DISCUSSION

The reproductive season for queen conch at LSI extends from mid-April to early October (Stoner et al. 1992); however, veligers

were collected only between the end of May and late September, with the vast majority occurring in a relatively narrow period between June and August. Although no correlation has been found between water temperature and reproductivity of queen conch at the study site (Stoner et al. 1992), seasonality of larval production associated with high summer temperature may be an adaptive strategy to shorten the time to metamorphosis and improve survivorship through the planktonic stage (Scheltema 1986). In laboratory culture, growth rates of queen conch veligers were highest in temperatures between 28 and 32°C, slowed at 24°C, and rapidly declined to near zero at 20°C (Stoner and Davis, unpubl. data). It is possible that production or types of phytoplankton food avail-

TABLE 1.

Density of *S. gigas* veligers (no. of veligers/10 m³) during the peak reproductive months (July and August) of 5 y at 13 stations near LSI, Bahamas.

Sites	Years				
	1988	1989	1992	1993	1994
Shark Rock flow field					
SR1	1.99 ± 0.33 (4)	2.41 ± 0.82 (4)	1.94 ± 0.70 (7)		
SR2*		4.20 ± 1.77 (5)	3.61 ± 1.00 (7)	1.61 ± 0.39 (7)	3.89 ± 1.22 (7)
SR3		2.31 ± 0.80 (4)			
SR4		2.52 ± 1.06 (4)	1.12 ± 0.37 (7)		
SR5	0.43 ± 0.02 (2)				
SR6*			1.98 ± 0.41 (7)	0.50 ± 0.34 (7)	
Children's Bay Cay flow field					
CBC1	1.90 ± 0.91 (4)				
CBC2*	0.98 ± 0.15 (3)		2.00 ± 0.46 (7)	1.81 ± 0.87 (7)	1.00 ± 0.42 (7)
CBC3	0.23 ± 0.06 (2)				
Middle Bank (MB)					
ephemeral nurseries		0.01 ± 0.01 (3)			
NPC*		2.56 ± 1.03 (3)			
NBC*	0.47 ± 0.18 (2)		0.68 ± 0.16 (7)	0.17 ± 0.08 (7)	
CHB*			0.16 ± 0.05 (7)		

Stations located in known nursery grounds are indicated with asterisks. Values are mean ± SE (n = number of sampling dates used to calculate the mean; two replicate tows were collected on each date).

able to larvae have affected the seasonality of reproduction in queen conch; however, recent measurements of chlorophyll *a* near LSI and throughout the Exuma Sound, in November 1993 and June 1994, have shown that concentrations of chlorophyll change little with season (A. Stoner, unpubl. data). The midsummer reproductive strategy in queen conch appears to be linked primarily to physical cycles in the environment, especially temperature and photoperiod, as suggested by Stoner et al. (1992), and not variation in phytoplankton biomass.

Because adult conch are abundant in 10- to 20-m depth all

along the Exuma Island chain (Stoner and Schwarte 1994, Stoner and Ray 1996) and because of the typically southeast to northwest alongshore drift (N. Smith, unpubl. data), it is likely that LSI receives larvae from spawning stocks to the south. Densities of midsize and late-stage larvae, therefore, are subject to events that are kilometers to tens of kilometers upstream in the Exuma Cays, whereas newly hatched veligers represent the local spawning stock. The significance of alongshore larval drift for the recruitment of dungeness crabs on the Pacific Coast of Washington was reported by McConnaughey et al. (1992).

A large literature has developed with respect to how larval fishes (Rowe and Epifanio 1994) and invertebrates (Heron et al. 1994) enter estuarine nursery areas by selecting particular strata on the flood tides (i.e., selective tidal stream transport, *sensu* Boehlert and Mundy 1988); however, there are very few data for molluscs (Mann 1988), and the mechanisms in nonestuarine systems may be quite different. Most likely, in the Exuma Cays, conch veligers are

TABLE 2.

Mean density of midsize (500–900 µm SL) and late-stage (>900 µm SL) veligers of *S. gigas* collected during three reproductive seasons in the Shark Rock flow field at nursery site SR2*.

Density of Veligers (no. · 10 m ⁻³)								
1992			1993			1994		
Date	Mid	Late	Date	Mid	Late	Date	Mid	Late
5/20	0	0	5/28	0	0	5/27	0	0.053
6/1	0	0	6/4	0	0	6/2	0	0
6/9	0.047	0	6/10	0	0	6/10	0	0.295
6/18	0.029	0	6/21	0.024	0	6/19	0.020	0
7/1	0.768	0.530	6/27	0	0	6/30	0	0
7/8	0.076	0.025	7/6	0.014	0	7/9	0	0.017
7/20	0	0	7/14	0	0	7/15	0.016	0
7/31	0	0	7/23	0.058	0.039	7/25	0.020	0
8/5	0	0	7/31	0.015	0	8/1	0	0
8/18	0.031	0.062	8/10	0.019	0.038	8/12	0	0
8/29	0	0.025	8/19	0	0.016	8/22	0	0
9/4	0	0	8/26	0.019	0	8/29	0	0
9/16	0	0.027	9/9	0	0	9/7	0.022	0
			9/16	0.041	0	9/14	0	0

Overall abundance data are shown in Figure 7.

TABLE 3.

Mean density of midsize (500–900 µm SL) and late-stage (>900 µm SL) veligers of *S. gigas* collected during two reproductive seasons in the Shark Rock flow field at the small nursery site SR6*.

Density of Veligers (no. · 10 m ⁻³)					
1992			1993		
Date	Mid	Late	Date	Mid	Late
7/1	0.080	0.135	6/21	0.016	0.100
7/8	0.050	0.030	7/23	0.019	0.038
9/16	0.025	0	7/31	0.084	0.084
			8/19	0	0.018
			8/26	0.028	0.048

Sampling dates with only newly hatched veligers were not included. Overall abundance data are shown in Figure 7.

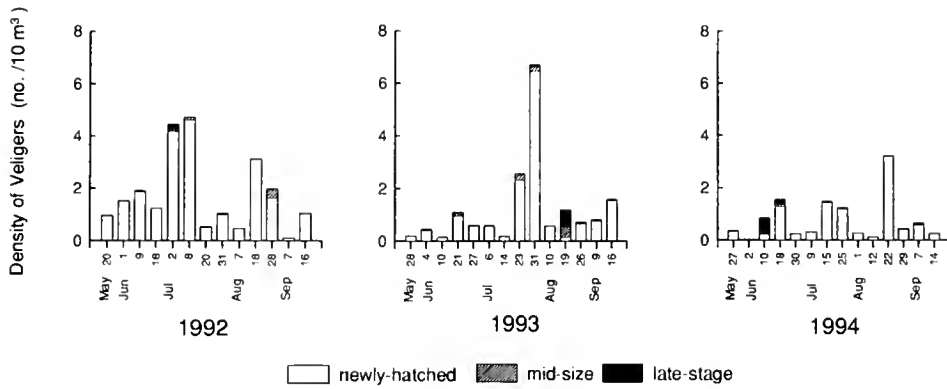


Figure 8. Mean density of queen conch veligers collected in the Children's Bay Cay nursery area (CBC2*) during three spawning seasons (1992, 1993, and 1994). Densities are reported by size category. Each column represents the mean for two plankton collections made on 13–14 sampling dates between May and September. Newly hatched veligers were 300–500 μm SL, midsize veligers were 500–900 μm SL, and late-stage veligers were >900 μm SL.

transported northwest on the alongshore current and then through passes between the islands on flood tide. Whether or not the larvae use behavioral processes to enter the inlets or remain on the bank is unknown; however, queen conch larvae migrate vertically over a few meters or tens of meters on a diurnal periodicity (Barile et al. 1994, Stoner and Davis 1997), and they may respond to salinity or temperature gradients that occur in the inlets. The most parsimonious explanation for the presence of large queen conch nurseries along the Exuma Cays island chain is the net bankward flow of shelf water (Smith and Stoner 1993).

Data from surface drifters indicate that once the veligers are drawn through the numerous inlets, they will be transported to nursery sites on the shallow bank (Stoner et al. 1996b). Plankton data presented in this study confirm the hypothesis that larval densities were high in the primary tidal streams, low in secondary

branches, and near zero in areas that do not receive regular incursions of water from the Exuma Sound. These new data provide an explanation for the observation that queen conch juveniles are absent from large, seemingly appropriate benthic habitats of seagrass outside major tidal currents in the Exuma Cays (Stoner et al. 1994). In fact, the highest larval densities occurred not only in association with the flow fields, but directly over the primary nursery ground at Shark Rock. Although significant densities of conch larvae were collected at sites farthest from the Exuma Sound (e.g., 5 km beyond the nursery), no midstage or late-stage larvae were ever found at locations beyond the nurseries. Comparable to our observations with queen conch, Field and Butler (1994) found that the postlarvae of spiny lobster (*Panulirus argus*) rarely settled beyond the emergent banks that ring Florida Bay. They concluded that the postlarvae were not regularly transported to the interior of

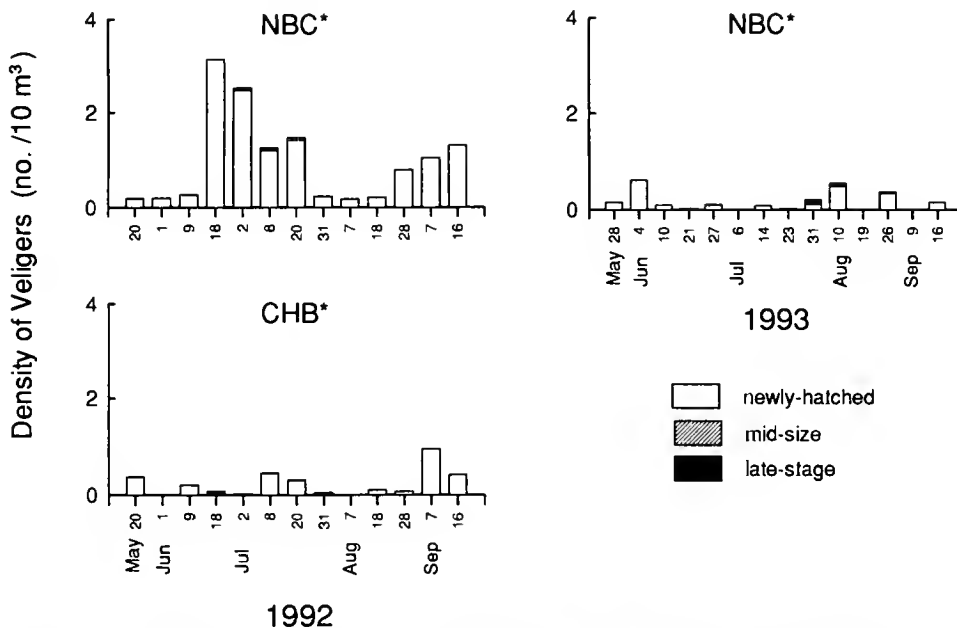


Figure 9. Mean density of queen conch veligers collected at the ephemeral nursery sites, Neighbor Cay (NBC*) and Charlie's Beach (CHB*), during two spawning seasons (1992 and 1993). Densities are reported by size category. Each column represents the mean for two plankton collections made on 13–14 sampling dates between May and September. Newly hatched veligers were 300–500 μm SL, midsize veligers were 500–900 μm SL, and late-stage veligers were >900 μm SL.

TABLE 4.

Mean density of midsize (500–900 μm SL) and late-stage (>900 μm SL) veligers of *S. gigas* collected during the three reproductive seasons in the Children's Bay Cay flow field at nursery site CBC2*.

Density of Veligers (no. \cdot 10 m ⁻³)								
1992			1993			1994		
Date	Mid	Late	Date	Mid	Late	Date	Mid	Late
5/20	0	0	5/28	0	0	5/27	0	0.019
6/1	0	0	6/4	0.021	0	6/2	0	0
6/9	0	0.024	6/10	0	0	6/10	0	0.609
6/18	0	0	6/21	0.103	0.031	6/19	0.081	0.181
7/1	0.073	0.176	6/27	0.015	0	6/30	0	0
7/8	0.105	0	7/6	0.016	0	7/9	0	0
7/20	0.019	0	7/14	0	0	7/15	0	0.022
7/31	0.022	0	7/23	0.206	0.018	7/25	0.022	0
8/5	0	0	7/31	0.176	0.059	8/1	0	0
8/18	0	0	8/10	0	0	8/12	0	0
8/29	0.337	0	8/19	0.411	0.629	8/22	0	0
9/4	0	0	8/26	0.046	0	8/29	0	0.020
9/16	0	0	9/9	0.018	0.036	9/7	0.023	0.045
			9/16	0.040	0	9/14	0	0

Overall abundance data are shown in Figure 9.

the bay, analogous to our findings for late-stage conch larvae on the Great Bahama Bank.

There is now considerable literature indicating that spatial and interannual heterogeneity in larval supply has an important influence on the settlement and recruitment of invertebrates (Yoshioka 1982, Gaines et al. 1985, Olmi et al. 1990, Bertness et al. 1992, Peterson and Summerson 1992, Martel et al. 1994) and fishes (Milicich et al. 1992). Sites with ephemeral juvenile conch populations had more sporadic densities of larvae than did the larger and more stable nursery sites near Shark Rock and Children's Bay Cay. For example, the ephemeral Tugboat Rock population (SR6*) had a lower mean density of veligers than the Shark Rock nursery (SR2*). SR6* lies in a secondary tidal branch associated with the inlet north of LSI and probably does not receive oceanic water on every tide, as does station SR2*. Low tidal current velocities would also reduce the flux of larvae to a site. Densities of larvae observed at NBC* and CHB* were typically much lower than

TABLE 5.

Mean density of midsize (500–900 μm SL) and late-stage (>900 μm SL) veligers of *S. gigas* collected during two reproductive seasons at the ephemeral nursery site NBC*.

Density of Veligers (no. \cdot 10 m ⁻³)					
1992			1993		
Date	Mid	Late	Date	Mid	Late
7/1	0.025	0.025	6/27	0	0.016
7/8	0.025	0.025	7/31	0.030	0.058
7/20	0.040	0	8/10	0.016	0.033
			8/26	0.016	0.017

Sampling dates with only newly hatched veligers were not included.

those at the more stable nursery sites, and densities of midstage and late-stage larvae were very low and erratic. It is likely, therefore, that population size and stability are related to the quantity and regularity of larvae arriving at a nursery. These results indicate that the importance of presettlement processes should be considered in the distributional ecology and management of queen conch populations.

Transplant experiments with juvenile conch near LSI (Stoner and Sandt 1992, Ray and Stoner 1994, Stoner et al. 1994) have shown that: (1) some habitats without resident conch can support juveniles, (2) conch nursery grounds are probably not saturated with juveniles in most years, and (3) recruitment probably limits the number of individuals in a nursery ground. We have also concluded that the settlement of conch larvae is not random. The Shark Rock nursery area possesses specific biological cues that induce a higher settlement rate than areas with seemingly similar general features both upstream and downstream from the nursery (Davis and Stoner 1994). Therefore, long-term queen conch nurseries, whether supporting large, stable populations of juveniles or small and ephemeral populations, are associated with a combination of important attributes: (1) hydrodynamic properties that supply and retain larvae, and (2) unique benthic characteristics that attract settlement of competent larvae and provide food and shelter for juveniles.

Although densities of larvae, particularly late stages, probably affect the distribution and abundance of juvenile conch in the nursery habitats, the abundance of early-stage larvae is undoubtedly influenced by the density or abundance of nearby spawners. Although few data exist for densities of queen conch larvae, some comparisons can be made on a regional scale. Densities of queen conch veligers were typically 1–2 larvae/10 m³ during the primary reproductive season near LSI, with some densities as high as 10 veligers/10 m³. In the Florida Keys, densities rarely exceeded 0.5 veligers/10 m³ between 1992 and 1994, even near important spawning sites (Stoner et al. 1996a, Stoner et al. 1997). The mean density of queen conch veligers in surface tows made by Posada and Appeldoorn (1994) in a July 1989 cruise along the islands of the eastern Caribbean Sea from Martinique to the Grenadines was 0.18 larvae/10 m³ (SD = 0.33, n = 19). The highest value in a single tow was 1.22 veligers/10 m³, found downcurrent from the important conch-producing banks of the Grenadines. Densities higher than those near LSI have been found only in the northern Exuma Sound, Bahamas. Stoner and Ray (1996) reported values commonly between 25 and 50 queen conch veligers/10 m³ in repeated samplings in the Exuma Cays Land and Sea Park during 1993 and 1994. Most larvae in collections just described have been newly hatched individuals; therefore, regional variation in observed densities is probably a direct function of spawning stock size or density in the surrounding areas. The density of adult conch in the Florida Keys was <3 individuals/ha in the primary habitats in 1989 (Glazer and Berg 1994), compared with 60–90 adults/ha in 10- to 20-m depth off LSI (Stoner and Schwarte 1994) and 100–250 adults/ha in the Exuma Cays Land and Sea Park (Stoner and Ray 1996).

The presence of queen conch in offshore waters of the Caribbean Sea (Posada and Appeldoorn 1994) and the genetic similarity of populations throughout the species' geographic range (Mitton et al. 1989) indicate that dispersal potential is high. Therefore, future research should emphasize the transport and supply of veligers on a local and regional scale. It will also be important to determine the relative importance of larval production and planktonic processes

in determining larval supply to nursery grounds (Meekan et al. 1993). Basic information—such as growth rates and length of larval life, survivorship in the water column, details of larval behavior, and physical oceanography—is needed to predict transport and to understand the interdependence of local populations. Experiments with natural phytoplankton foods (Olson and Olson 1989) and analysis of statolith rings, which represent daily growth rates in gastropod veligers (Grana-Raffucci 1989, Bell 1993, M. Davis, unpubl. data), should be particularly valuable. Larval transport studies will be key to the preservation of the most important spawning populations and to the rehabilitation of this, and other, overexploited species.

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ABUNDANCE AND DISTRIBUTION OF QUEEN CONCH VELIGERS (*STROMBUS GIGAS* LINNE) IN THE CENTRAL BAHAMAS. II. VERTICAL PATTERNS IN NEARSHORE AND DEEP-WATER HABITATS

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ABSTRACT The vertical distribution of queen conch veligers was investigated in three different habitats in the central Bahamas: (1) over an island shelf reproductive site (18 m deep) near Lee Stocking Island, Exuma Cays, Bahamas; (2) in a tidal channel leading from the reproductive ground to nursery grounds on the bank (8 m deep); and (3) in the deep-water basin of Exuma Sound. At the reproductive site, early-stage larvae were most abundant near the surface (0- to 1-m depth) during calm periods. Concentrations were highest in the deepest layer sampled (16 m) during periods of moderate swell and wave height. Day/night variation was not consistent, and vertical distribution appeared to be more closely associated with surface conditions than time of day. In the tidal channel, conch veligers were most abundant in the upper 1 m during day, night, and crepuscular hours. Lower concentrations of veligers were found in the neuston and at 3- and 6-m depths. Midstage (500–900 μm shell length) and late-stage ($>900 \mu\text{m}$) larvae were relatively rare at the nearshore reproductive and tidal channel sites. At the deep-water site, offshore in the Exuma Sound, queen conch veligers of all stages (mostly midstage and late stage) were collected at all depths sampled, from the surface to 100 m. However, the larvae were concentrated in the upper mixed layer, which was 25–30 m deep. During the day, 83% of the larvae were found in the 0.5- to 5.0-m-deep layer, well above the pycnocline. Low concentrations were found at all other depths, including the upper 0.5 m. At night, the larvae were evenly distributed between 0.5- and 30-m depths. These data corroborate laboratory experiments showing positive phototaxis in queen conch larvae up to very high light levels and disruption of phototaxis at night when the light cue is weak. The fact that few larvae were found below the thermocline, even at night, suggests that they are adapted to remain in warm surface waters, where growth is maximum.

KEY WORDS: Bahamas, oceanography, *Strombus gigas*, vertical migration

INTRODUCTION

Vertical distribution and diel migration are common phenomena in marine zooplankton, including invertebrate larvae. Depth regulation is influenced by exogenous factors such as presence of predators (Bollens and Frost 1989, Forward 1988, Neill 1990), distribution of food (Enright 1977, Dagg 1985, Daro 1988), changes in salinity (Sulkin 1984, Mann et al. 1991), and intensity of light (Forward 1976, Kaartvedt et al. 1987, Swift and Forward 1988). Physical parameters, such as tidal currents, may also affect the vertical position of larvae in the water column and can be responsible for the retention and transport of larvae to favorable settlement habitats (Hill 1991, Olmi 1994).

The queen conch, *Strombus gigas*, is an important fisheries' species in the Caribbean region, and many populations have suffered overexploitation during the past two decades (Berg and Olsen 1989, Appeldoorn 1994). For effective fisheries and conservation management of the species, it is necessary to determine larval sources and their dispersal mechanisms to preserve key spawning populations and to predict juvenile recruitment. The basic life history of juvenile and adult queen conch is well studied (Randall 1964, Brownell and Stevely 1981, Stoner et al. 1994), but the natural history of the planktotrophic conch veliger is poorly known. Data on the geographic distribution and abundance of veligers have been published only recently (Stoner et al. 1992, Posada and Appeldoorn 1994, Stoner et al. 1994). Although preliminary analyses of vertical distribution revealed that queen conch veligers were most abundant at the surface (1 m deep) during the

day (Chaplin and Sandt 1992, Stoner and Davis 1997), they have been collected from depths as great as 30 m in deep-water habitats of the eastern Caribbean Sea (Posada and Appeldoorn 1994).

Recent laboratory and field mesocosm experiments showed that queen conch veligers are positively phototactic up to very high light levels and that this taxis decreases with age (Barile et al. 1994). Conch veligers swam toward the water surface in both light and dark conditions, suggesting that negative geotaxis is also important for orientation in this species.

A detailed analysis of the vertical distribution of queen conch veligers in the field is reported for the first time in this study. Collections were made in waters near Lee Stocking Island (LSI), Exuma Cays, Bahamas, an area characterized by large spawning populations (Stoner and Schwarte 1994, Stoner and Ray 1996). We provide data on day and night vertical distribution and abundance of veligers at three different locations—a spawning site on the island shelf, a tidal channel between reproductive and nursery habitats, and deep oceanic water. These field studies contribute information on stimuli that control vertical distribution in the veligers and will be critical in modeling larval transport and recruitment potential.

MATERIALS AND METHODS

Vertical Sampling in the Shelf and Channel Locations

Stratified vertical sampling for veligers was conducted at a well-studied reproductive site (RS) and a tidal channel station (SR1), both near LSI in the central Bahamas (Fig. 1). Detailed descriptions of the study site and tidal circulation were provided in earlier publications (Stoner et al. 1994, Stoner et al. 1996, Stoner and Davis 1997). RS was approximately 1 km to the east of LSI, on the island shelf in Exuma Sound (ES), where a spawning popu-

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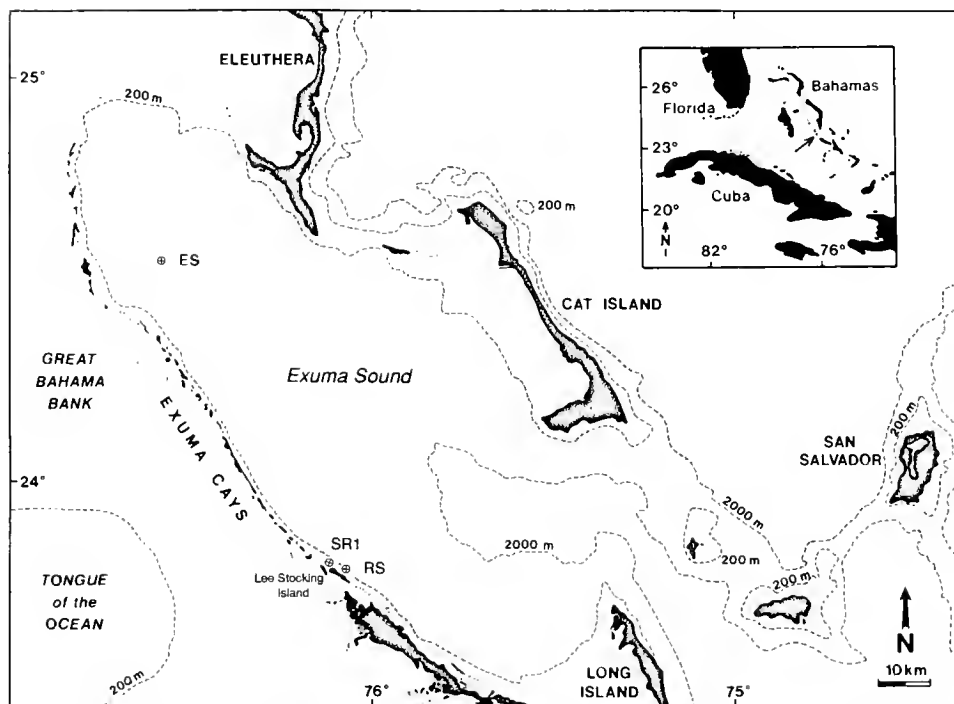


Figure 1. Map showing three sites where vertical plankton collections were made in the ES area of the central Bahamas. RS was the reproductive site on the island shelf immediately offshore from LSI. SR1 was in the tidal channel north of LSI. ES was a deep-water site in the northern sound.

lation occupies a sand- and algae-covered platform 18 m deep (Stoner and Sandt 1992). In 1990, collections were made at four depths at RS: at the air-sea interface (neuston), in the upper 1 m (surface), at 8-m depth (midwater), and at 16-m depth (near-bottom).

A simple conical net (0.5 m in diameter, 2.5 m in length) with 202- μ m mesh was used to collect all larval stages, including newly hatched veligers, which have a maximum shell dimension of approximately 300 μ m. Nets were generally towed with a small boat (6 m) at 1.0 m/sec for 10–15 min in the downwind and alongshore direction (usually northwest). A calibrated General Oceanics flow-meter suspended off-center in the mouth of the net was used to estimate water volume sampled, typically 200–250 m³. The neuston layer was sampled by towing the net with the middle of the ring at the air-sea interface. Tows at depth did not use an opening/closing mechanism, but the nets were lowered and raised through the water column while the boat was stationary. Lead weights suspended from the net ring allowed for towing at 8- and 16-m depth and for quick lowering to avoid depth contamination. The position of the near-bottom net was maintained by permitting the weight, suspended 2 m below the ring, to touch the bottom periodically. The depth of the midwater net was estimated by wire angle and by eye in the very clear water. Replicate tows ($n = 2$) were made for each depth on each sample date. Samples were collected in June, July, and August, with seven vertical series collected during the day (1100–1400 h) and two complete series collected for day and night (2300–0300 h) comparisons. Wind speed and direction, wave height, and cloud coverage were estimated and recorded at the time of each collection.

The second site, SR1, was located on the bank in the tidal channel north of LSI (Fig. 1). Conch larvae are carried through this inlet to a large, well-studied nursery ground west of LSI (Stoner et al. 1996, Stoner and Davis 1997). High current velocities on flood

tide (to 2.0 m/sec) made it possible to collect plankton with fixed nets moored at three different depths. In 1989 and 1990, a taut mooring was rigged with a large concrete block and surface bouys. Nets identical to those described above were attached to the mooring at 3- and 6-m depth using SCUBA and were retrieved 30 min later. The nets were kept closed by the diver during deployment and retrieval. At the same time, surface water (upper 1 m) was sampled using a net suspended from an outrigger on a boat that was anchored adjacent to the mooring. In 1990, the neuston layer was also sampled using the outrigger mechanism. Because of high current velocities at the surface, the duration of sampling was reduced to 20 min. The average volume of water sampled at each depth was 160 m³. Collections were made during the middle of the flood tide, when the tidal current was strongest and when veliger abundance was highest (Stoner and Davis 1997). In 1989, vertical collections for veligers at SR1 were made for three day/night series in July and August; one net was set at each depth on the mooring on each sampling date. In 1990, 14 vertical collections were made during the day (0700–1900 h), during the night (2300–0300 h), and during crepuscular hours (approximately 0500 and 2100 h) between June and August. Collections were replicated ($n = 2$) for each of the four depths on each sampling date.

Vertical Sampling at the Deep-Water Site

Vertical distribution of conch veligers was examined to depths of 100 m in the deep-water basin of ES during June 1994. The 18-m R/V *Shadow* was maintained on station 90 km northwest of LSI and 18 km east of the Exuma Cays (24°30'N, 76°34.5'W) (Fig. 1). This site is approximately 1,600 m deep and was chosen because sampling at the site in 1993 yielded high concentrations of conch larvae (primarily midstage and late stage) (Stoner, unpubl. data).

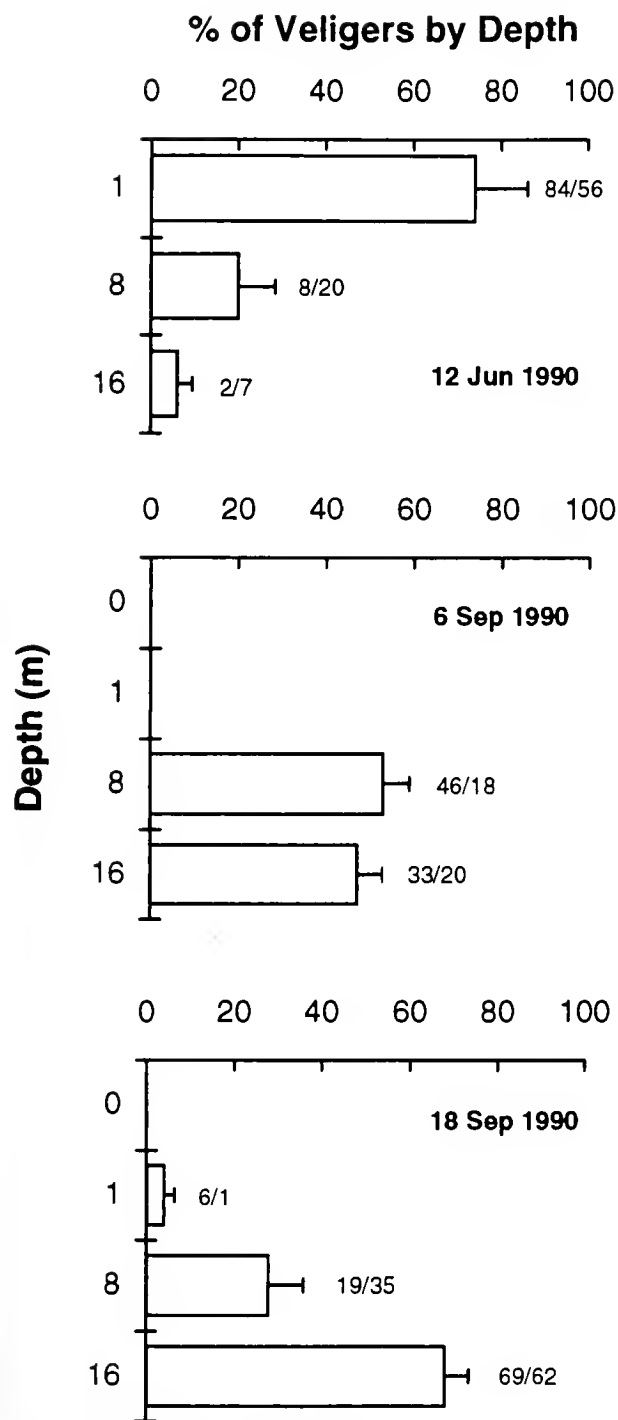


Figure 2. Percentage of veligers at each of four depths during daytime collections made at the RS on two dates in 1990 (mean \pm SE, $n = 2$ tows at each depth). Values next to the error bars represent the actual number of veligers collected in Tow 1 and Tow 2.

Collections were made during the day (1100–1500 h) on June 23 and the following night (2200–0300 h). Nets were similar to those described earlier, except that they were equipped with General Oceanics double-trip, opening-closing mechanisms for deployment on hydrographic wire. While maintaining a vessel speed of 1.0 m/sec, nets were set over the side from a long boom where they were unaffected by the ship's wake. Oblique tows were made

TABLE 1.

Size and density of veligers collected at the RS in 1990 during three daytime sampling dates at three depths.

Location	Veliger Stage		
	Newly Hatched	Mid	Late
June 12			
Surface (1 m)	4.17 \pm 0.71	0.14 \pm 0.14	0
Midwater (8 m)	1.05 \pm 0.31	0	0
Near-bottom (16 m)	0.34 \pm 0.13	0	0
September 6			
Surface (1 m)	0	0	0
Midwater (8 m)	2.20 \pm 0.68	0	0
Near-bottom (16 m)	1.81 \pm 0.29	0	0
September 18			
Surface (1 m)	0.04 \pm 0.04	0	0.26 \pm 0.09
Midwater (8 m)	1.93 \pm 0.41	0	0
Near-bottom (16 m)	4.81 \pm 0.13	0.04 \pm 0.04	0

No veligers were collected in the neuston. Newly hatched veligers were 300–500 μ m SL, midsize veligers were 500–900 μ m SL, and late-stage veligers were >900 μ m SL. Values are mean number of veligers/10 m³ (\pm SE) ($n = 2$ tows).

over depth intervals of 0–0.5, 0.5–5, 5–10, 10–30, 30–60, and 60–100 m. The nets were sent to the greatest depth of the desired sampling interval (calculated from wire angle), opened at depth, slowly raised through the water column to the upper depth limit over a period of approximately 15 min, and then closed and retrieved. Nets set for the two shallowest depths were left open. To maintain precise control of sampling intervals, nets were set one at a time on the wire. All tows were replicated twice in random order for each time period. Profiles of temperature and salinity were made to 150-m depth with a SeaBird CTD at the beginning and end of both day and night collections.

Identification and Staging of Veligers

In 1989 and 1990, plankton samples were sorted live within 4 h after collection. In 1994, samples were preserved in 5% buffered formalin immediately after collection and were sorted within 5 mo. Veligers of *S. gigas* were identified by comparison with laboratory-reared specimens and by using shell features described by Davis et al. (1993). Sorting and measuring techniques were summarized by Stoner and Davis (1997). Conch larvae were classified as newly hatched (300–500 μ shell length [SL]), midsize (500–900 μ m), or late stage (>900 μ m), most of which were competent for metamorphosis.

Data Analysis

Larval densities were standardized by converting counts to number of veligers/10 m³. Means of replicate tows were calculated and, in some cases, were used to calculate the mean density of veligers for a particular time of day or depth. Because of temporal variation in absolute larval densities, vertical abundance data were converted to percentages of larvae at each depth. Veliger densities were low at SR1 in 1990. When fewer than four veligers were collected on a particular date, these samples were not considered in the analysis. The χ^2 goodness of fit test was used to analyze percent distribution for the 1990 data from SR1, with the null hypothesis that veliger distribution was homogeneous over depth.

One-way analysis of variance, followed by Tukey's multiple comparison test, was used to determine if the depth distribution and abundance of veligers in the deep-water ES site were significantly different between day and night. The proportional data were arcsine transformed to reduce heteroscedasticity.

RESULTS

RS

The daytime vertical distribution of veligers was examined to 16-m depth (near-bottom) at the offshore RS in 1990 (Fig. 2). Large temporal variation in the vertical distribution of veligers was associated with changing weather and sea conditions. On June 12, a calm, overcast day (3.6 m/sec wind; 0.5-m sea height; no swell), conch veligers were concentrated (74%) in the upper 1 m of the water column. On September 6 and 18, in association with turbulent sea conditions, the depth distributions were distinctly deeper. The majority of veligers were collected at 8 and 16 m, with few to none collected in the neuston and at 1-m depth (Fig. 2). Fewer than 10 veligers were collected on each of the four other sampling dates

(June 7, July 12, August 8, and September 3), and results are not shown. On these dates, winds were 6–10 m/sec and seas were 1–2 m. The percentage of veligers at different depths showed no direct correlation with wind speed ($r < 0.2$; $p > 0.5$) but may have been inversely related to sea height, which was not measured precisely. Conch veligers were never collected in the upper 1 m of the water column when waves were breaking into white caps in winds > 8 m/sec.

The majority of the veligers found at RS were newly hatched. Midsized veligers were found only on June 12, and September 18, 1990, just four veligers in the surface water and one near the bottom, respectively (Table 1). Late in the reproductive season (September 18, 1990), seven late-stage veligers (0.26 veligers/10 m³) were found in the surface water.

No clear diurnal vertical pattern of distribution was discernible from the day/night vertical collections of veligers made during two relatively calm 24-h periods at RS (< 3 m/sec wind; < 0.3 -m sea height; no swell) (Fig. 3). At night in June, most veligers (78%) were collected in surface water (1-m depth). During midday, the abundances of veligers were relatively similar at 1- and 8-m depth.

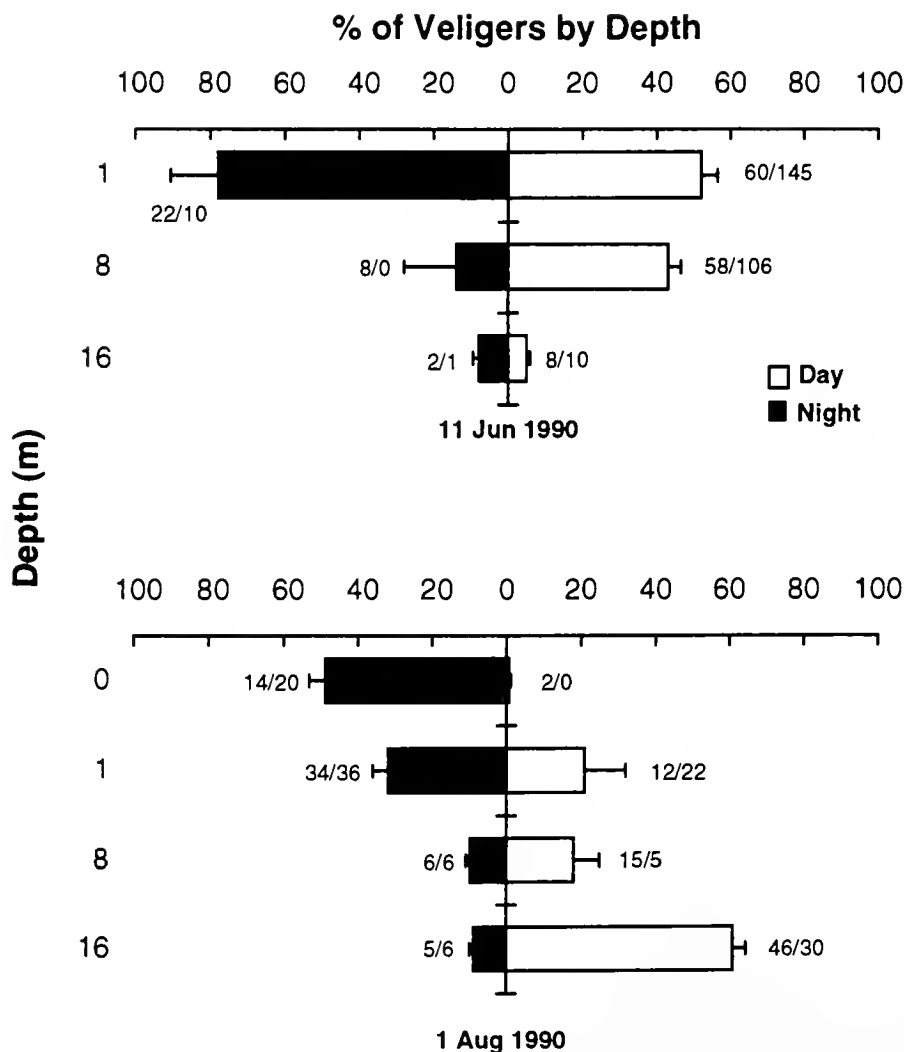


Figure 3. Percentage of veligers at each of four depths during the day and night at the RS on three dates in 1990 (mean \pm SE, $n = 2$ tows at each depth). Values next to the error bars represent the actual number of veligers collected in Tow 1 and Tow 2 and do not necessarily parallel the results in percentages, which were standardized per unit of water volume sampled.

In August, a high percentage (49%) of veligers was collected in the neuston at night, whereas in the daytime, the majority of veligers (61%) were near the bottom. The overall density of veligers was higher in the daytime than at night, with the highest density (7.44 veligers/10 m³) estimated for daytime surface waters on June 11, 1990 (Table 2).

Most veligers collected at RS in day/night series were newly hatched (Table 2). Only one midsize veliger was collected, and this individual was in surface water during the day on August 1, 1990. Slightly more abundant late-stage veligers were collected on several occasions during the day and night in the neuston, surface, and near-bottom waters. Relatively low densities of midstage and late stage (≤ 0.17 veligers/10 m³) (Table 2) precluded conclusions about vertical or day/night distribution in these stages in the island shelf habitat.

Channel Site (SR1)

Veligers collected in the inlet in 1989 were distributed throughout the water column, apparently the result of surface conditions. In the daytime on July 7 and August 7, light wind (2.5–3.6 m/sec) and mild sea conditions (<0.5-m height) resulted in the highest percentages of veligers in surface waters (Fig. 4), approximately twice the densities estimated for 3- and 6-m depths (Table 3). In contrast, in the daytime on July 24, more turbulent surface conditions (5–7 m/sec winds, 0.6- to 1.3-m sea height) resulted in a lower percentage of veligers in the upper water column than at 3- and 6-m depths. At night, the numbers of veligers collected were very low, and vertical patterns were inconsistent (Fig. 4). On July 6, 1989, veligers were found exclusively at 3 m; on July 23, they were found at the surface and at 3 m; and on August 6, the distribution was relatively uniform. The higher percentage of veligers near the surface on the night of July 23, compared with the low percentage of veligers during the following day, probably resulted from weather conditions that were calmer at night than during the day.

On all three sample dates in 1989, the density of veligers was consistently higher in the day (0.93–5.46 veligers/10 m³) than at night (0–0.25 veligers/10 m³) at all three depths (Table 3). SL did not vary from day to night or by depth. All veligers were newly hatched, 417–450 μ m.

More intensive sampling resulted in a clearer depth distribution of veligers in 1990 (Fig. 5) than in 1989. The percentages of veligers were always highest in the surface 1.0-m depth during all three time periods—day, crepuscular, and night. Densities in the neuston layer were highest at night but were always lower than in the water column immediately below (Fig. 5b). The depth distributions were significantly different from homogeneous in collections made both with and without neuston samples ($\chi^2_{0.01,3} > 40.0$, and $\chi^2_{0.01,2} > 24.6$, respectively). The majority of the 727 veligers collected in the 1990 surveys were newly hatched. Three late-stage veligers were collected at 1-m depth on July 29, and two midsize and one late-stage veliger were collected in the same upper layer on August 27 (Tables 4 and 5). Differences in absolute concentrations of veligers at SR1 between day and night observed in 1989 were not apparent in 1990.

Deep-Water Site

A distinct vertical distribution of queen conch veligers and an indication of diel variation were evident in the oceanic water of the ES during the relatively calm sampling period (wind < 5 m/sec; sea < 1.0 m) (Fig. 6). As observed in the nearshore habitats, only a small percentage of veligers were located in the neuston during both day and night; however, night concentrations in the neuston were higher than during the day (Fig. 6). Depth differences in the distribution of veligers were significant in the daytime [$F_{(5,6)} = 27.34$, $p < 0.001$]. The highest percentage (83%) of veligers was observed in the surface layer (0.5–5 m) (Tukey's test, $p < 0.002$); abundances at all other depths were low and not different ($p > 0.646$) (Fig. 6). Even though the majority of veligers occurred between 0.5- and 30-m depth at night, the abundance of veligers did not vary significantly with depth [$F_{(5,6)} = 1.20$, $p = 0.407$], probably because of the low number of replicate samples ($n = 2$ at each depth). Veligers were collected as deep as 100 m during both day and night; however, >95% of all veligers were collected above 30 m.

Very few early-stage larvae were collected at the deep-water site in ES (Fig. 7), in contrast to the mostly early-stage larvae collected at RS and SR1. The highest recorded mean density of late-stage veligers (1.56 veligers/10 m³) was found in night tows at 5- to 10-m depth; this was more than twice the maximum value

TABLE 2.

Size and density of veligers collected at the RS in 1990 during two day/night sampling series at four depths (neuston = 0–0.5 m; surface = 1 m; mid-water = 8 m; and near-bottom = 16 m).

Location	Day			Night		
	Veliger Stage			Veliger Stage		
	Newly Hatched	Mid	Late	Newly Hatched	Mid	Late
June 1						
Surface	7.44 \pm 2.38	0	0	1.00 \pm 0.37	0	0.03 \pm 0.03
Mid	5.83 \pm 1.27	0	0	0.29 \pm 0.21	0	0
Bottom	0.61 \pm 0.05	0	0	0.10 \pm 0.02	0	0
August 1						
Neuston	0	0	0.17 \pm 0.17	2.38 \pm 0.29	0	0
Surface	0.70 \pm 0.40	0.02 \pm 0.02	0.11 \pm 0.06	1.46 \pm 0.01	0	0.05 \pm 0.05
Mid	0.79 \pm 0.29	0	0	0.52	0	0
Bottom	2.5 \pm 0.36	0	0	0.40 \pm 0.10	0	0.04 \pm 0.04

Newly hatched veligers were 300–500 μ m SL, midsize veligers were 500–900 μ m SL, and late-stage veligers were >900 μ m SL. Values are mean number of veligers/10 m³ (\pm SE) ($n = 2$ tows).

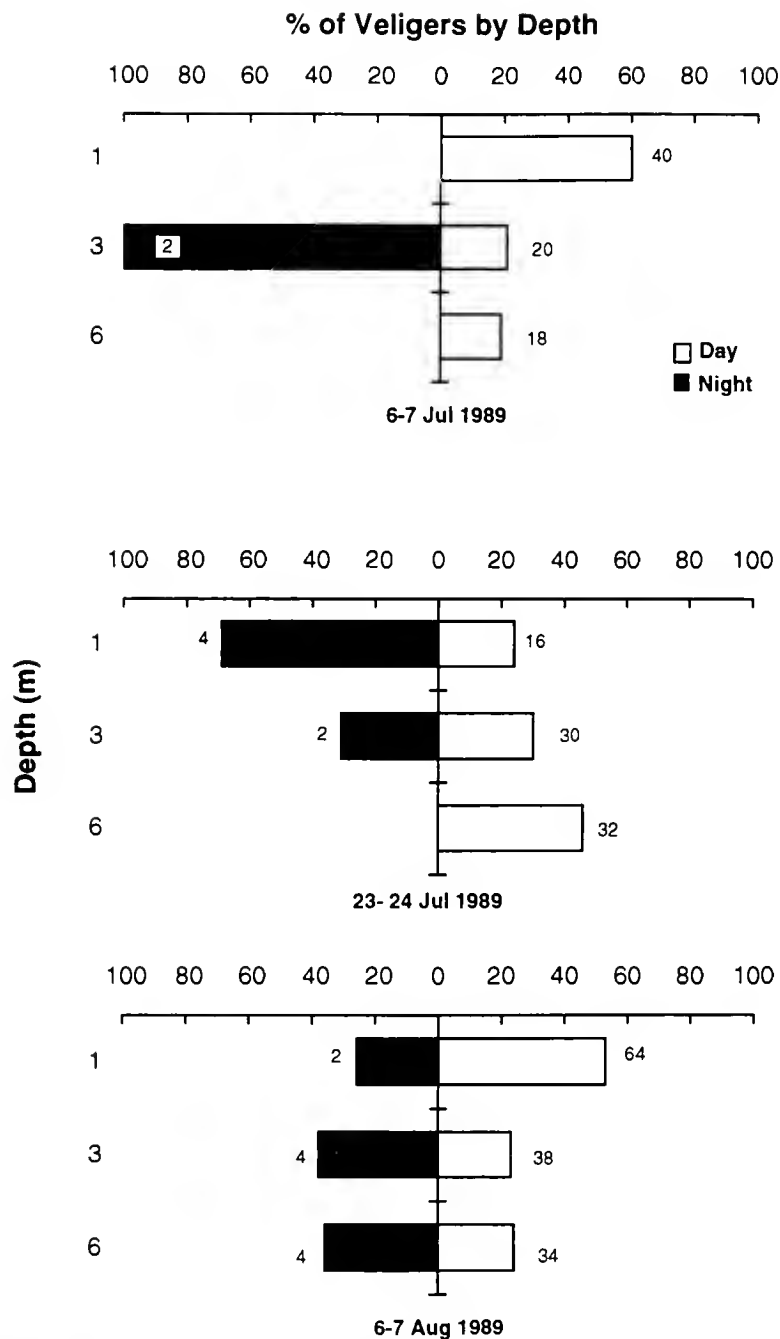


Figure 4. Percentage of veligers at each of three depths during the day and night in the tidal channel (SR1) on three dates in 1989 ($n = 1$ tow at each depth). Values next to the bars represent actual number of veligers collected in the tow.

estimated for day collections (0.68 veligers/ 10 m^3 at $0.5\text{--}5\text{ m}$) (Fig. 7) and six times the highest value found at the RS (0.26 veligers/ 10 m^3) (Table 1). The vertical and diurnal patterns of density in midsize veligers were similar to those of late-stage larvae (Fig. 7). Midsize larvae were very abundant (2.0 veligers/ 10 m^3) during the day in $0.5\text{--}5\text{ m}$ depth.

Results from day and night CTD casts at the deep-water site were virtually identical; therefore, only one representative is shown (Fig. 8). A well-defined upper mixed layer to $25\text{--}30\text{ m}$ depth was clearly indicated by the strong discontinuities in temperature, salinity, and density. A lens of water with slightly lower salinity than the surface layer was centered at approximately 35 m .

The surface layer where most queen conch veligers were found had a temperature of 29°C and a salinity of approximately 37 ppt.

DISCUSSION

In a preliminary investigation of vertical distribution in queen conch veligers, Chaplin and Sandt (1992) concluded that the veligers move upward during the day and downward at night, a reverse diurnal vertical migration. This conclusion was based primarily on the low abundance of veligers detected in surface waters during the night. In conflict with this reverse migration hypothesis, Barile et al. (1994) found that queen conch larvae of all stages

TABLE 3.

Density of veligers collected in the tidal channel (SR1) in 1989 during three day/night sampling series at three depths.

Location	Density of Veligers (no./10 m ³)	
	Day	Night
July 6-7		
Surface (1 m)	5.46	0
Midwater (3 m)	1.93	0.08
Near-bottom (6 m)	1.70	0
July 23-24		
Surface (1 m)	0.93	0.23
Midwater (3 m)	1.14	0.09
Near-bottom (6 m)	1.71	0
August 6-7		
Surface (1 m)	5.32	0.17
Midwater (3 m)	2.32	0.25
Near-bottom (6 m)	2.40	0.23

All veligers were newly hatched (300–500) μ m SL). Values are for one net set at each sampling depth.

migrated upward at night in 3-m-deep field mesocosms. On the basis of several laboratory experiments, they concluded that depth distribution in this species is affected by positive phototaxis, negative geotaxis, and negative phototaxis at very high light intensity. Similar behavior has been observed for larvae of the gastropod *Phestilla sibogae* (Miller and Hadfield 1986). Whether an endogenous rhythm is entrained in the vertical movements of queen conch veligers is still unresolved. Barile et al. (1994) suggested that vertical distribution in the species is associated with particular light levels rather than specific rhythms.

Variation in vertical distribution patterns in the field (this study) shows that observations from the laboratory should be ex-

trapolated to the field with care. Under calm surface conditions, the majority of larvae (all stages) were found in near-surface waters during the daytime in all of the habitats sampled (e.g., upper 1.0 m in shallow water; 0.5- to 5-m layer in deep water). This was congruent with laboratory and mesocosm indications of positive phototaxis and upward movement during the day (Barile et al. 1994). However, the clear pattern of diel vertical migration expressed in mesocosms was not always observed in the field. For example, day, night, and crepuscular distribution patterns were not different in intensive sampling of the tidal inlet in 1990. Conversely, the depth of the veligers appeared to be directly related to wind velocity; this was particularly evident in the open-water environment over the RS. A similar effect of wind-induced turbulence on vertical distribution has been observed for bivalve veligers (Raby et al. 1994). Although some conch veligers moved to the upper 0.5 m at night, as would be predicted by the laboratory and mesocosm experiments (i.e., with negative geotaxis), a large number also dispersed to greater depths (to 30 m) in the deep-water habitat. This appears to be associated with the absence of a light cue and a relatively weak negative geotaxis.

Veligers were associated with surface waters, but very few were found in the neuston during the day. This could be a simple response to veliger preference for specific light levels or avoidance of particular wavelengths. Many marine zooplankton, including echinoid plutei (Pennington and Emlet 1986), avoid potentially harmful wavelengths in the upper water column (Damker et al. 1980). Barile et al. (1994) found that queen conch larvae were higher in laboratory water columns and field mesocosms when ultraviolet wavelengths were filtered out; however, the differences with and without filtration were not statistically significant, and the exact reasons for avoiding the brightly lit surface remain unknown.

Although queen conch veligers were found as deep as 100 m in the ES, densities below the pycnocline (30 m) were very low. Similar surface-layer associations have been observed for a variety

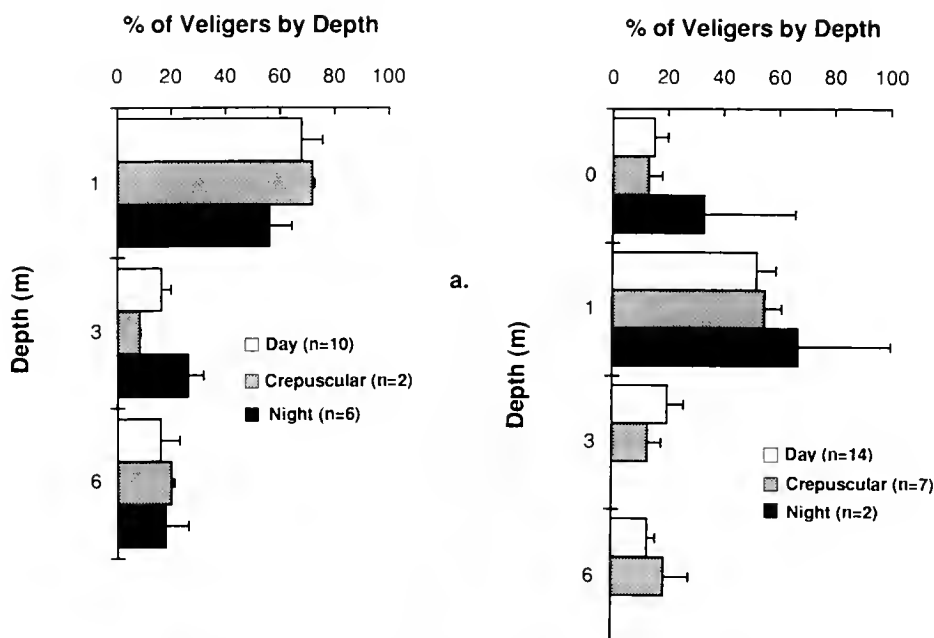


Figure 5. Percentage of veligers collected over depth during day, crepuscular, and night periods in the tidal channel (SR1) in 1990 (mean \pm SE). Samples with fewer than four veligers were eliminated from the analysis. (a) Dates with three depths sampled. (b) Dates with four depths sampled, including the neuston layer.

TABLE 4.

Density of veligers collected in the tidal channel (SR1) in 1990 during multiple day, night, and crepuscular samplings at three depths.

Sampling Period	Density of Veligers (no./10 m ³)		
	Average (\pm SE)	Minimum	Maximum
Day (n = 10 tows)			
Surface (1 m)	1.19 \pm 0.52	0.15	5.69
Midwater (3 m)	0.28 \pm 0.09	0	0.88
Near-bottom (6 m)	0.25 \pm 0.10	0	0.92
Crepuscular (n = 2 tows)			
Surface (1 m)	1.51 \pm 0.97	0.54	2.47
Midwater (3 m)	0.18 \pm 0.12	0.06	0.29
Near-bottom (6 m)	0.40 \pm 0.24	0.16	0.64
Night (n = 6 tows)			
Surface (1 m) ^a	1.29 \pm 0.45	0	3.17
Midwater (3 m)	0.74 \pm 0.40	0.12	2.69
Near-bottom (6 m)	0.45 \pm 0.22	0	1.30

All veligers were newly hatched (300–500 μ m SL), except in one night surface tow.^a

^a On July 29, three late-stage veligers were found in one two (0.33 veligers/10 m³).

of invertebrate larvae (Young and Chia 1987) including other molluscs (Tremblay and Sinclair 1992). Many marine zooplankton (Dagg 1985, Daro 1988), including bivalve larvae (Raby et al. 1994), are known to migrate vertically according to distribution of food. However, concentrations of chlorophyll in ES were essentially uniform to 250 m during June 1994, and there was no indication of a deep chlorophyll maximum (unpubl. data). Of course, it is possible that there were depth-related differences in the phytoplankton community or in their nutritional value to queen conch larvae. Investigations on the relationship between natural phytoplankton foods and the growth and nutrition of conch larvae have just begun (Davis, in press, Davis et al. 1996).

TABLE 5.

Density of veligers collected in the tidal channel (SR1) in 1990 during multiple day, night, and crepuscular samplings at four depths.

Sampling Period	Density of Veligers (no./10 m ³)		
	Average (\pm SE)	Minimum	Maximum
Day (n = 14 tows)			
Neuston (0–0.5 m)	0.17 \pm 0.44	0	6.3
Surface (1 m) ^a	1.51 \pm 0.37	0.06	5.48
Midwater (3 m)	0.42 \pm 0.13	0	1.83
Near-bottom (6 m)	0.46 \pm 0.12	0	1.32
Crepuscular (n = 7 tows)			
Neuston (0–0.5 m)	0.13 \pm 0.06	0	0.40
Surface (1 m)	0.61 \pm 0.16	0.11	1.4
Midwater (3 m)	0.16 \pm 0.06	0	0.35
Near-bottom (6 m)	0.13 \pm 0.04	0	0.22
Night (n = 2 tows)			
Neuston (0–0.5 m)	0.25 \pm 0.25	0	0.50
Surface (1 m)	0.85 \pm 0.60	0.25	1.44
Midwater (3 m)	0	0	0
Near-bottom (6 m)	0	0	0

All veligers were newly-hatched (300–500 μ m SL), except in one daytime surface tow*.

* On August 27, two midsize veligers (0.13 veligers/10 m³) and one late-stage veliger (0.10 veligers/10 m³) were found in one tow.

One of the most important adaptive advantages conferred on queen conch larvae living in the upper mixed layer is the high growth rate associated with high temperature. In the laboratory, growth and survivorship in conch larvae are maximum at 28–29°C (Davis 1994). Mixed layer temperatures in the ES during the summer spawning season are typically 28–30°C, with temperature decreasing rapidly to approximately 25°C at 100-m depth; therefore, larvae remaining above the thermocline probably have the highest growth rates and the shortest time exposed to pelagic predators.

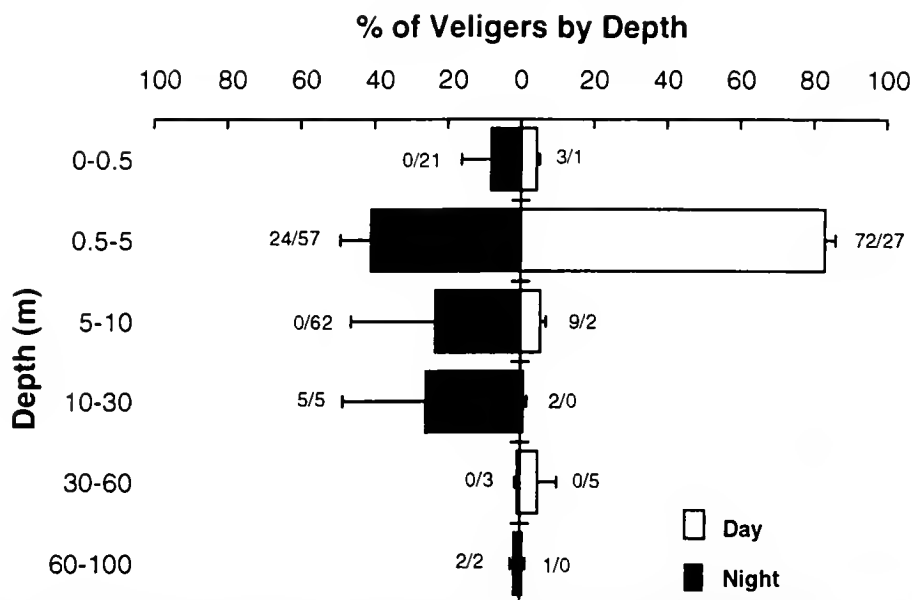


Figure 6. Percentage of veligers at each of six depths during the day and night at the deep-water site in ES in June 1994 (mean \pm SE, n = 2 tows at each depth). Values next to the error bars represent the actual number of veligers collected in Tow 1 and Tow 2.

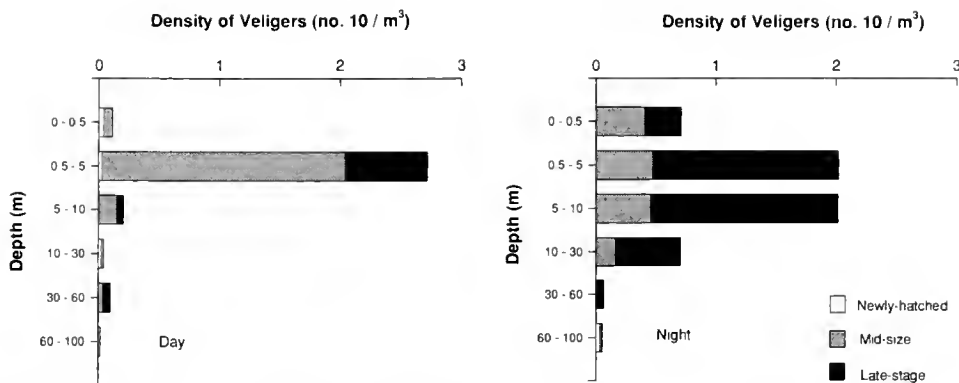


Figure 7. Mean density and size composition of veligers collected at the deep-water site in ES in June 1994 ($n = 2$ tows per sampling date). Newly hatched veligers were 300–500 μm SL, midsize veligers were 500–900 μm SL, and late-stage veligers were >900 μm SL.

Ontogenetic shifts in vertical position and migration are commonly observed in marine invertebrate larvae (Forward and Costlow 1974, Chia et al. 1984, Power 1989), but no obvious age-related changes in vertical distribution were detected in queen conch larvae. Similarly, larvae of sea scallops, *Placopecten magellanicus*, also demonstrate no ontogenetic shift in vertical distribution (Tremblay and Sinclair 1990).

Larval supply can be an important variable in determining the distribution, abundance, and year-class strength of queen conch (Stoner et al. 1994, Stoner and Davis 1997). Because this study shows that estimates for veliger abundance are strongly influenced by vertical movements, we provide the following guidelines for routine surveys. (1) Sampling can be made with minimal sampling gear because conch larvae are near the surface (0.5–1 m) during the daytime in relatively calm weather. However, because they disperse to the greatest range of depth at night and sink to greater

depths during rough weather, we recommend that sampling be restricted to daylight hours when wind is <6 m/sec (10–12 knots) and seas are <1 m. Where depth permits, we recommend making oblique tows to 5-m depth. (2) Sampling in tidal areas is particularly problematic. Our studies in the tidal inlet north of LSI have shown that veliger abundance is strongly influenced by tidal period (Stoner and Davis 1997) as well as by time of day. Vertical turbulence in a tidal channel is also dependent on current velocity; therefore, the vertical position of zooplankton in a tidal field and their associated transport potential will depend on sampling time and the locomotory capacities of the organisms (Smith and Stoner 1993). Preliminary analyses for sampling strategy will be critical in tide-influenced areas. (3) Net mesh size should be chosen according to the purpose of sampling. A 202- μm mesh must be used to collect newly hatched queen conch veligers, whereas a mesh size of 333 μm is appropriate for the collection of late-stage ve-

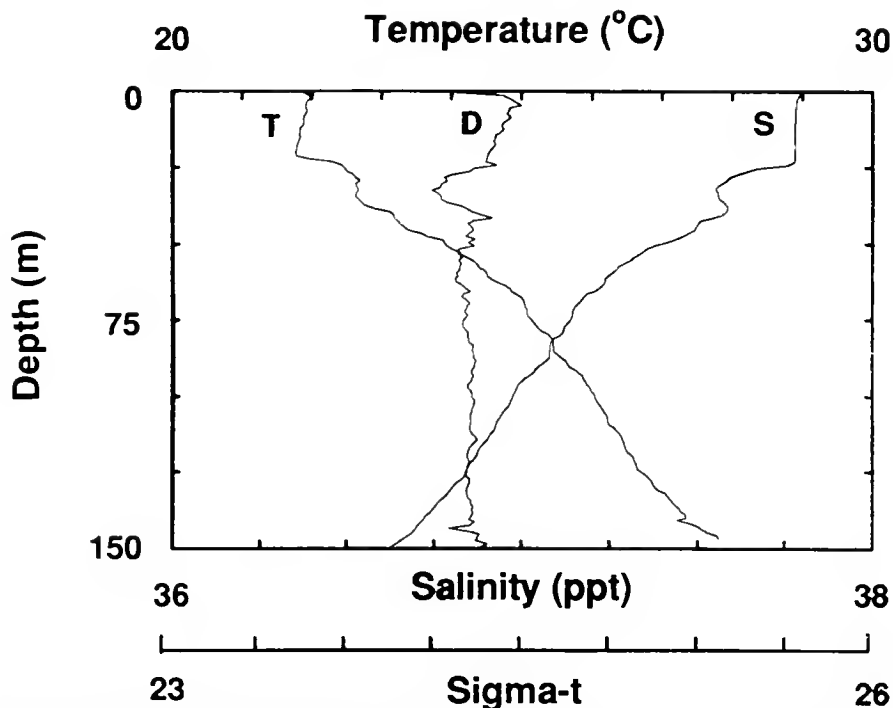


Figure 8. Temperature (T), salinity (S), and density (D) profiles during plankton sampling at the deep-water site in ES in June 1994. Four CTD profiles taken over the 16-h sampling period were identical.

ligers. A larger mesh size allows for towing through a larger water volume without clogging the net mesh and for easier sorting. (4) The size of the net mouth will depend on the tow vessel and the density of the target species. We have found that 0.5-m-diameter nets are useful in general surveys for conch larvae, even in sites with low larval density. Larger nets (0.75 or 1.0 m) are particularly valuable when the less abundant late-stage veligers are being sampled with larger mesh. (5) Conch veligers should be preserved in a buffered (pH 7.5–8.5) 5% formalin–seawater mixture, and if the sample contains a large amount of organic material, its pH should be checked and maintained every month. The shells of early-stage conch veligers are damaged quickly in low-pH solutions, making positive identification to species difficult if not impossible.

Knowledge of larval transport and supply is critical to the understanding of recruitment processes and sound fisheries' management in species with pelagic larvae. The development of realistic models for transport will depend on good information on the vertical distribution and movements of larvae relative to both physical and biological characteristics of the environment. It is clear from

this study that vertical distribution in queen conch veligers is influenced by the basic behavioral traits of the larvae, their responses to physical gradients, which vary over both time and space, and their swimming capabilities in the upper water column, which is frequently turbulent.

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LABORATORY SPAWNING AND JUVENILE REARING OF THE MARINE GASTROPOD: SPOTTED BABYLON, *BABYLONIA AREOLATA* LINK 1807 (NEOGASTROPODA: BUCCINIDAE), IN THAILAND

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ABSTRACT Laboratory spawning and juvenile rearing of the marine gastropod, *Babylonia areolata* L., is described. Adults were collected from wild stock in the inner eastern Gulf of Thailand. *B. areolata* laid individual, moderately transparent, vasiform egg capsules and firmly attached them to the sand substratum by a long narrow stalk. The egg capsules average 21.43 mm long and 9.57 mm wide. Each adult female spawned 47 capsules with 851 eggs per capsule. Average eggs were 425.70 μ m in diameter. Embryonic development occurred inside the egg capsules for 7 days. On Day 8, larvae (720.40 μ m) hatched through the apical opening of the egg capsule into water. Metamorphosis from the free swimming planktotrophic larvae to benthic juveniles took 18 days. Newly settled juveniles were 1.52 and 1.16 mm in shell length and width, respectively. The monthly average growth increments of juveniles were 4.26 per month in length and 2.28 g/mo in weight. Average survival rate was 94.08%.

KEY WORDS: *Babylonia areolata*, growth, survival, spawning, rearing, egg capsules

INTRODUCTION

The spotted babylon, *Babylonia areolata*, commonly known as Hoy wan in Thailand, is a carnivorous marine benthic gastropod of the order Neogastropoda, family Buccinidae (Habe 1965). The shell is thick with a high, pointed apex, and the body whorl is patterned, with round brownish patches on the white shell background. The species is abundant all year and inhabits the muddy sand bottom, usually less than 10–20 m in depth. Literature on *B. areolata* is limited (Munprasit and Wudthisin 1988, Singhgraiwan et al. 1989). Other studies on the biology of *Babylonia spirata*, *Babylonia zeylonica*, and *Babylonia lutos* have been recently done in Hong Kong and India (Thirumavalavan 1987, Morton 1990, Shanmugaraj et al. 1994, Ayyakkannu 1994, Raghunathan et al. 1994, Patterson et al. 1994, Raghunathan and Ayyakkannu 1995).

Babylonia is an important marine resource harvested from the natural local beds. The fishery has developed as a by-product of sand crab (*Portunus pelagicus*) harvests. *Babylonia* harvest has recently declined in traditional areas, particularly in the larger size classes. In response to the decreased production was a resulting increase in both demand and price. The price of *babylonia* of 5.0–6.5 cm shell length is about 7.2 and 10.0 US\$ per kilogram in seafood markets and restaurants, respectively. In recent years, *babylonia* mariculture has been proposed as a means of increasing supply. Continuous yearly exploitation of *babylonia* may result in the depletion of local stocks. The declining stocks and interest in aquaculture prompted this study on spawning, larval development, and juvenile rearing of *B. areolata*.

MATERIALS AND METHODS

Broodstock Preparation

One hundred adult *B. areolata* (Fig. 1) were obtained from the littoral region of Rayong Province, in the inner eastern Gulf of

Thailand (Fig. 2), by local fishermen. These broodstocks were then transported to Sichang Marine Science Research and Training Station, about 40 km from the collection site. The animals were held in 2.0 \times 1.0 \times 0.8 m spawning tanks supplied with flow-through seawater (10 L/min). Salinity and temperature ranged from 26 to 29 ppt and 28 to 29°C, respectively. A 10-cm layer of fine sand was provided as substratum. The animals were fed twice daily with fresh meat of carangid fish, *Selaroides leptolepis*. The adult snails were acclimatized for 5–10 days to spawn naturally in the laboratory.

Hatching

After spawning, egg capsules were collected and rinsed with 1- μ m filtered seawater. In order to remove the foulings contaminating the surface of egg capsules, the capsules were soaked in wellwater for 30–60 sec. The capsules were then placed in plastic baskets of 1-cm mesh size and submerged in 1.5 \times 0.5 \times 0.3 m hatching tanks containing 1- μ m filtered and gently aerated seawater. Water was replenished daily until hatching. The egg capsules per snail and eggs per capsule were counted and measured for morphology studies. Egg capsules containing eggs and embryos in different stages of development were sampled daily, preserved in 5% neutral formalin, and examined microscopically to evaluate intracapsular development.

Larval Development

After hatching, the newly hatched planktonic veligers were collected with a 200- μ m nylon mesh sieve and rinsed with 1- μ m filtered, ambient seawater three times. These veligers were transferred to 1.5 \times 0.5 \times 0.3 m rearing tanks containing 1- μ m filtered, ambient, continuously aerated seawater. The initial stocking density was 10,000 larvae per liter. Larvae were fed twice daily with



Figure 1. Breeders' *B. areolata* (5.7 cm shell length) used in this study.

20×10^6 cells mL^{-1} of mixed unicellular microalgae consisting of a 1:1 ratio of *Chlorella* spp. and *Tetraselmis* spp. Water was changed every 2 days, and the rearing tank was cleaned with 3 ppt chlorine concentration for 10 min and rinsed with wellwater

two to three times. The development of larvae was monitored during the first hour; larvae were then sampled at 1, 2, 3, 4, 8, 12, 14, 16, and 24 h after hatching and at 24-h intervals during the following 2 wk. Growth of at least 20 larvae per sample was periodically examined under microscope over the 4 wk. The larvae were also sampled and counted every 3 days to calculate survival rate.

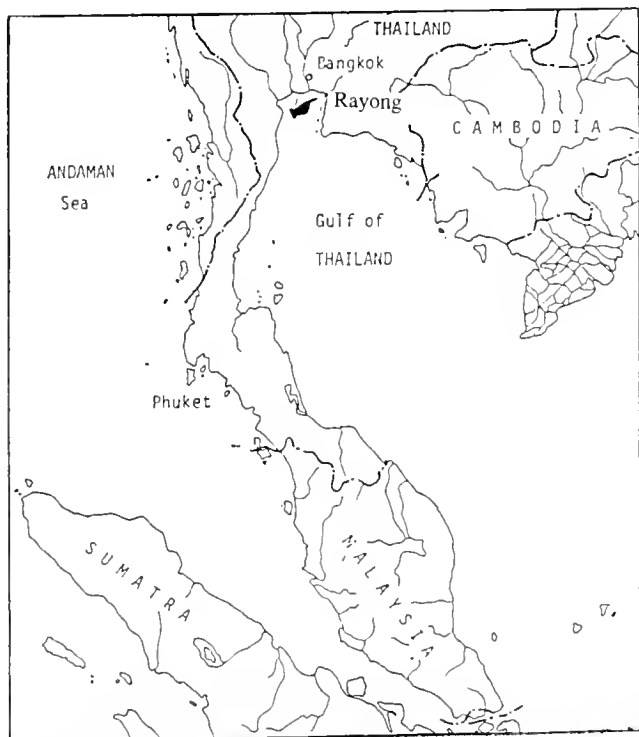


Figure 2. Fishing ground of Rayong province, in the eastern Gulf of Thailand.

Juvenile Rearing

Larvae set on the bottom of the larval rearing tanks. The settled juveniles were transferred into $1.5 \times 0.5 \times 0.5$ m rearing tanks. The tanks were supplied with flow-through seawater (10 L/min) and gently aerated. At an average shell length of 16.50×2.58 mm, the juveniles were transferred to duplicate $1.0 \times 1.0 \times 0.5$ m rearing tanks containing a 10-cm layer of fine sand. The initial stocking density was 100 individuals per m^2 . After transfer, the food was changed from unicellular microalgae to chopped carangid fish (*S. leptolepis*). Snails were fed twice daily at 9:00 PM and 17:00 AM. Food was offered until the animals stopped eating. The length and weight were measured at monthly intervals over a 6-mo period. The absolute growth rates and their standard deviation were calculated from average increments in shell size and total weight per month. The number of dead individuals was recorded in each tank at monthly intervals, and an average monthly survival rate was calculated.

Geometric mean regression analyses of shell dimensions (length and width) were calculated to determine morphological relationships (Wolff and Garrido 1991).

$$TL = a + bWi,$$



Figure 3. Egg capsules of *B. areolata* before (right) and after (left) hatching.

where TL and Wi are any two shell dimensions of shell length and width (mm), and a is the intercept and b is the slope of the regression line.

Similarly, the length-weight relationship was determined using the logarithmically transformal allometric equation:

$$\log Wt = \log a + b \log TL$$

where W represents body weight (g) and Lx stands for any one shell dimension (cm).

RESULTS

Spawning Behavior

Adult spawning stocks of *B. areolata*, with average shell length of 5.69 ± 0.3 cm (SD: $n = 35$), spawned naturally during March and April 1996. Spawning took place during early morning. Most egg capsules were individually attached to the sand substratum by a long, narrow stalk. Newly laid egg capsules were moderately

TABLE 1.

Morphology of broodstock, egg capsules, and number of eggs per capsule of *B. areolata* under hatchery conditions.

No.	Broodstock Sizes			Egg Capsules Spawned			Capsules Containing Eggs	
	Length ^a (cm)	Width ^b (cm)	Weight ^c (gm)	Number (capsule)	Length ^d (mm)	Width ^e (mm)	Egg No. per Capsule	Egg Diameter (μ m)
1	6.13	3.57	40.70	56	23.30	10.00	1133	420
2	5.83	3.36	40.10	54	21.10	11.20	1041	415
3	6.05	3.36	42.10	46	20.0	11.10	933	446
4	5.94	3.46	40.50	57	21.10	10.00	940	465
5	5.46	3.33	38.80	44	22.20	9.20	807	438
6	5.81	3.35	44.10	42	20.00	8.20	493	422
7	5.52	3.24	33.20	53	22.20	9.00	733	389
8	5.11	3.94	32.50	22	23.30	10.00	1253	428
9	5.38	2.41	38.30	37	19.59	8.00	507	394
10	5.74	3.33	30.90	56	21.60	9.00	673	440
X \pm SD	5.69 \pm 0.32	3.67 \pm 0.38	38.12 \pm 4.42	46.70 \pm 11.08	21.43 \pm 1.32	9.57 \pm 1.08	851.30 \pm 255.15	425.70 \pm 23.16

^a Measured from the maximum distance between the tip of the spire and the siphonal canal.

^b Measured from the greatest distance to the opposing side of the body whorl.

^c Whole weight.

^d Measured from the distance between the beginning of the stalk and the tip of capsule.

^e Measured from the greatest distance to the opposing side of the capsule.

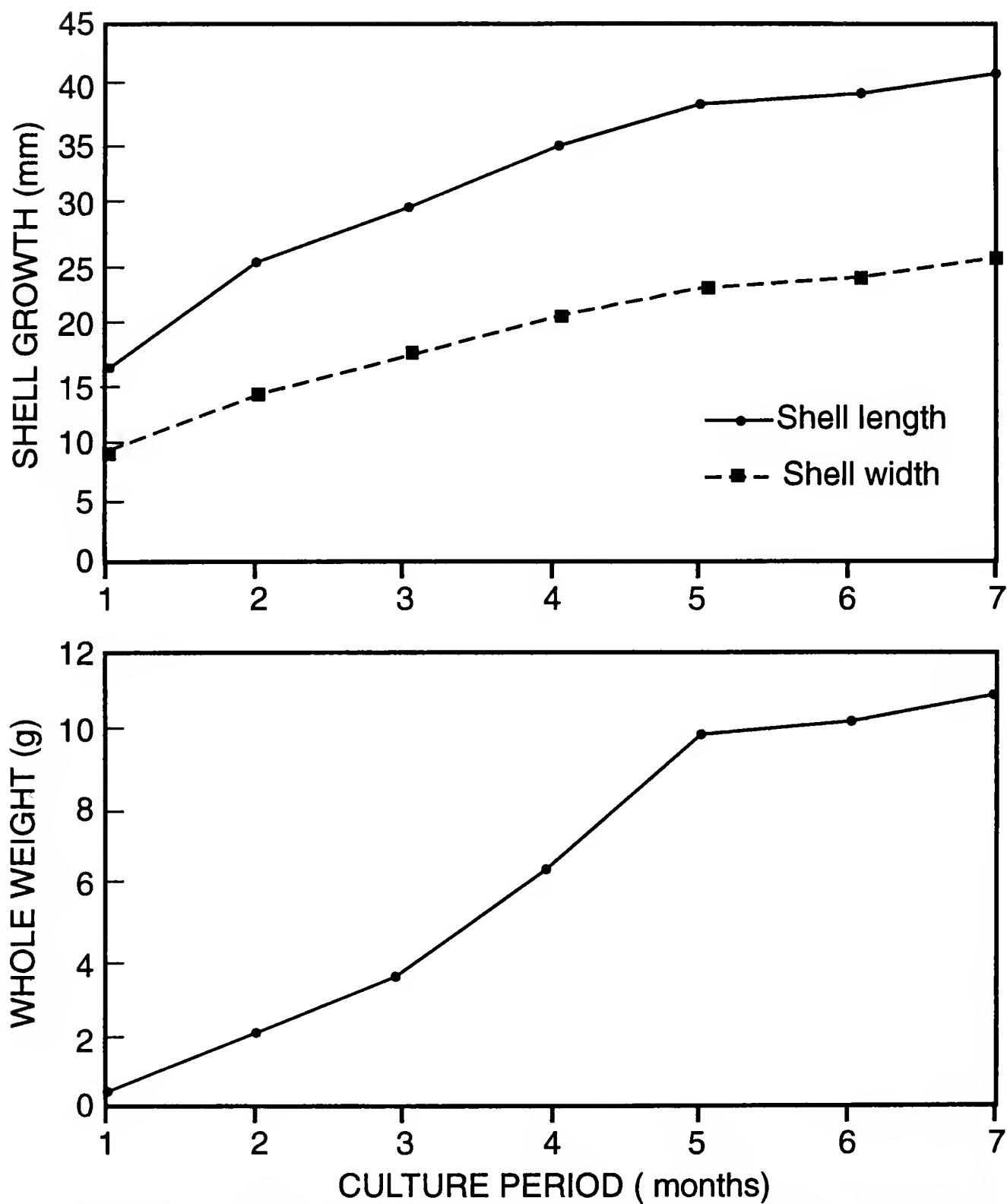


Figure 4. Average monthly growth in length and weight of juvenile *B. areolata* reared under hatchery conditions over a 6-mo period.

transparent and vasiform in shape. The capsules were broad at the apex and narrower toward the base, and each capsule possesses a short stalk (peduncle) that is cemented to the substrate (Fig. 3). The eggs are visible and suspended in albuminous fluid inside the capsule. Egg capsules averaged 21.43 ± 1.32 mm in width, 9.57 ± 1.08 mm in length, 11.40 ± 0.85 mm in peduncle length, and 10.54 ± 0.52 in escape aperture length (SD; $n = 20$). An average female babylonia (5.69 cm long) spawned 46.7 ± 11.08 egg capsules (SD; $n = 12$; range = 22–57). The average egg number per capsule was 851.30 ± 255.15 (SD; $n = 15$; range = 493–1,133), and the average egg diameter was 425.70 ± 23.16 μ m (SD; $n = 20$). *B. areolata* fecundity averaged 39,146 eggs per individual. The morphometric comparisons of broodstock, egg capsules, and egg numbers of *B. areolata* are presented in Table 1.

Larval Development

The larvae developed from single cell to early veliger stage inside egg capsules during the first 7 days. The veliger larvae hatched through the apical opening into the water column within 8 days after spawning. Veliger larvae were hatched at 28–30 ppt salinity and 26–28°C water temperature. The hatching rates were 89.00%. The newly hatched veligers had a transparent, thin shell and two large, lobed velums. The average shell length of veligers was 720.40 ± 1.52 μ m (SD; $n = 20$). After hatching, veliger larvae were positively phototactic and planktotrophic. At Day 9, the velar lobes became enlarged, with shells visible, and the larvae were about 870 μ m long. The velar lobes degenerated, and the foot became obvious on about Day 13. At this stage, the larvae were about 1,450 μ m long. By Day 16, the siphonal canal, tentacles, and eyes had become visible and the velar lobes were almost disintegrated. The larvae settled to the bottom at about 1,540 μ m. On Day

16, the presence of a foot and swimming near the bottom were the first indications that the larvae were competent to settle. Metamorphosis was completed by Days 18–20, and the juveniles averaged $1,520 \pm 1.64$ μ m long and $1,160 \pm 1.36$ μ m wide (SD; $n = 20$). Larvae metamorphosed and settled in the absence of substratum. Average growth increment was 84.44 μ m in shell length per day, and survival was 2.4%. During the period of settlement, heavy mortality occurred because the newly settled juveniles continually crawled out of the water and died as a result of desiccation.

Juvenile Rearing

The average growth and survival rates of juvenile *B. areolata* reared under hatchery conditions is represented in Figure 4. Average monthly growth of juvenile *B. areolata* (both length and weight) rapidly increased over the first 4 mo and, thereafter, gradually decreased. The average monthly growth increments were 4.26 mm/mo in length, 2.80 mm/mo in width, and 2.28 g/mo in weight. At the end of the experiment, juveniles had reached an average total length, width, and body weight of 42.10 ± 8.97 mm, 26.18 ± 5.90 mm, and 14.24 ± 4.13 g (SD; $n = 50$), respectively (Figure 5).

Monthly survival rate increased during the first 3 mo, and then no further mortality was observed. The average monthly survival rate was 94.08 over the 6 mo of juvenile culture (Fig. 6).

The equations describing the relationships between shell length, width, and weight were as follows:

$$TL = 3.1200 + 1.5073 Wi \quad (r^2 = 0.9660)$$

$$\log W = -7.0923 + 0.4005 \log TL \quad (r^2 = 0.8498)$$



Figure 5. Juvenile *B. areolata* of 40 mm shell length at the end of the study.

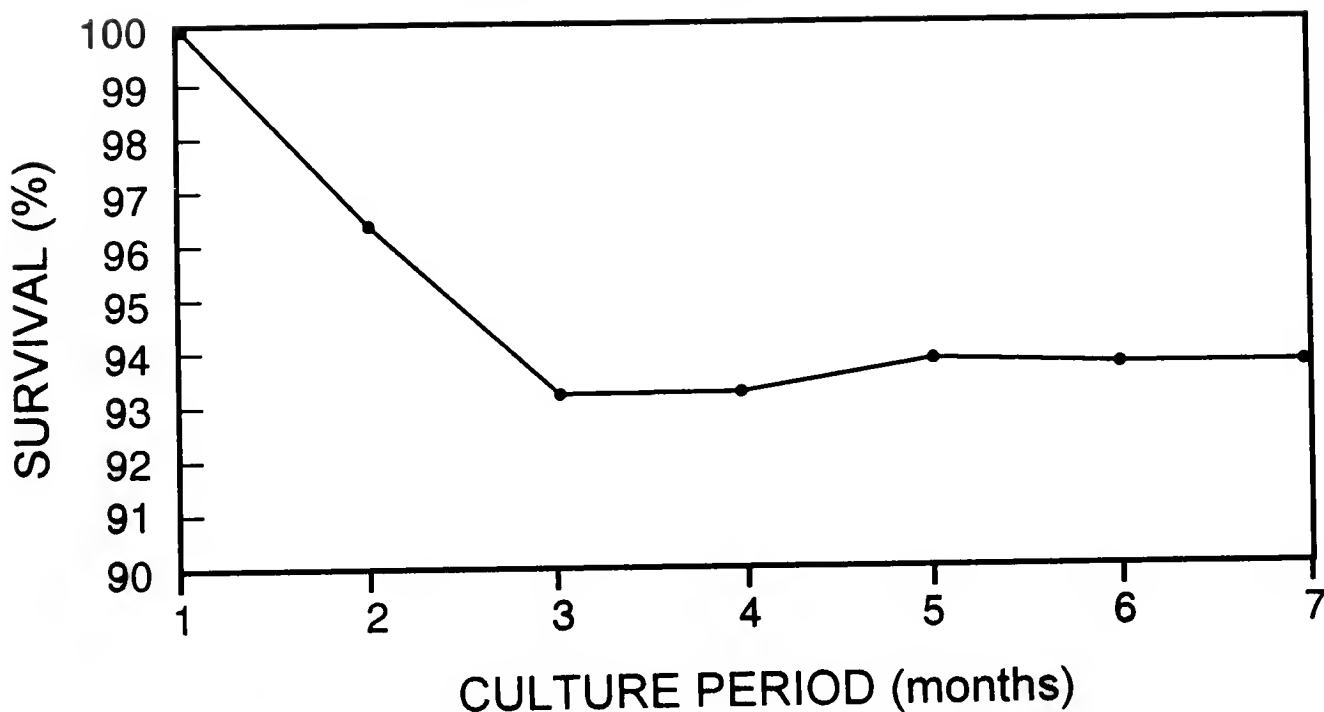


Figure 6. Average monthly survival of juvenile *B. areolata* reared under hatchery conditions over a 6-mo period.

DISCUSSION

B. areolata was spawned under hatchery conditions during March and April 1996. Egg capsules were individually attached to sand substratum with a long, narrow stalk. Each female spawned an average of 41 egg capsules, with 1,052 eggs per capsule. Spawning of *B. areolata* was similar to that of the spiral babylonia, *B. spirata*, but differed from that of the muricid gastropod, *Chicoreus ramosus*. Shanmugaraj et al. (1994) reported that *B. spirata* laid 24–35 transparent, vasiform egg capsules attached to the substratum. Each capsule contained about 900 eggs in a jelly-like fluid. The veliger larvae hatched and metamorphosed within 10 and 19 days after hatching, respectively. Kannapiran (1994) found that *B. spirata* had between 28 and 41 egg capsules. The highest fecundity was 36,900 eggs per snail per year. Bussarawit and Ruangchua (1991) and Nugranad (1992) reported that egg capsules of the muricid gastropod, *C. ramosus*, are laid in a compact mass of multiple capsules firmly attached to the substratum. The egg capsules were moderately translucent, vase shaped, tough, and creamy-white in color, and they measured about 16.0 mm in height and 3.7 mm wide. The escape aperture is placed centrally on top of the egg capsules, and the mean number of larvae per capsule was 341.

Larval development of *B. areolata* was similar to that of *B. spirata*, but differed from that of *C. ramosus*. Shanmugaraj et al. (1994) reported that larvae of *B. spirata* hatched through apical openings within 10 days after spawning, and they completely metamorphosed into juveniles 1.9 mm long within 19 days. In *C. ramosus*, hatching occurred 25–28 days after spawning; the newly hatched larvae were about 580 μ m and larvae 1.4 mm long metamorphosed within 3 wk. Survival ranged from 1.75 to 99.5% after hatching (Nugranad 1992, Bussarawit and Ruangchua 1991, Nugranad et al. 1994). Heavy postset mortality of *B. areolata* took place because the newly settled juveniles crawled out of the water, desiccated, and died. Similar phenomena were observed in *B. spi-*

rata (Shanmugaraj et al. 1994) and busyconine whelk, *Busycon carica*, (Kraeuter et al. 1989, Castagna and Kraeuter 1994).

Average monthly growth rate of juvenile *B. areolata* was 4.06 mm/mo in length and 0.97 g/mo in weight. The growth rate of *B. areolata* was greater than that of *B. spirata* or *C. ramosus*. Raghunathan et al. (1994) reported that the average growth of *B. spirata* showed a gradual increase from 2.95 to 3.00 to 3.55 to 3.86 cm in shell length and 6.4 to 7.8 to 11.10 to 14.10 g in total weight over a 10-mo period. Patterson et al. (1995) reported average growth rates of 1.22 mm and 0.05 g/day for *B. spirata* fed oyster and crab. In contrast, juveniles of *C. ramosus* showed average growth increments of 2.60, 9.26, 4.27, and 1.01 mm/mo in shell length at 2, 5, 8, and 12 mo, respectively (Nugranad et al. 1994). Kraeuter et al. (1989) reported that average growth rate of knobbed whelk, *B. carica*, was 14.40 mm/y for the first 10 y of life in laboratory conditions.

This study showed that *B. areolata* has characteristics that may make it a potentially valuable aquaculture species. It exhibited a fast growth, market size being reached in 10 mo, using relatively simple hatchery techniques. Additional studies to manipulate gonadal development and culture technology of this species are necessary. These should include methods to improve growth and survival of larvae and juveniles in both nursery and growout systems and to evaluate the costs of scaling up the culture conditions to commercial levels.

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SEASONAL STUDIES OF FILTRATION RATE AND ABSORPTION EFFICIENCY IN THE SCALLOP *CHLAMYS FARRERI*

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ABSTRACT Seasonal studies of filtration rate, retention efficiency, and absorption efficiency in the native scallop *Chlamys farreri*, a major component of shellfisheries and aquaculture species in northern China since the 1970s, were carried out four times between September 1993 and May 1995 in Sungan Bay, Shandong, China. This is the first time that the feeding physiology of *C. farreri* has been studied. The experiments were carried out semi-in situ using a running seawater system into which natural seawater was pumped directly from the nearshore off-bottom of the experimental site and no other food was added. The variation of particle organic matter, total particle matter, and chlorophyll *a* (chl *a*) in the natural seawater of the experimental site was 1.09–4.40 mg/L, 3.79–17.66 mg/L, and 1.98–4.89 µg/L, respectively. The exponents (*b*) in the allometric equation ($FR = aW^b$) of filtration rate as a function of dry tissue weight in different seasons varied in a narrower range and averaged 0.43, whereas the elevations (*a*) varied in a wider range between 1.33 and 4.35, and the order of elevations from the highest to the lowest was September, May, April, and November. This indicated that the seasonal patterns in the filtration rate of this scallop were correlated with seawater temperature. Measurement of the absorption efficiency showed no differences among individuals of different sizes, but there were differences among different seasons. November (63.1%) and April (60.7%) had higher mean absorption efficiencies than did September (44.6%). It seemed that the higher the chl *a* content of the seston and the more favorable the environmental condition, the higher the scallop's absorption efficiency.

KEY WORDS: *Chlamys farreri*, filtration, absorption, retention, semi-in situ

INTRODUCTION

As one of the most important shellfish species in northern China, the annual production of the native scallop, *Chlamys farreri* Jones & Preston, amounted to 400,000 metric tons in 1994. The initiation of intensive aquaculture of this species in recent years has led to negative effects on the scallops such as depressed growth rates and increased mortality, mainly caused by overcrowding, which ultimately reduced the food available to the scallops. One way to resolve this problem is to estimate the carrying capacity for scallop culture in the culture area and adjust the culturing density accordingly. In order to model carrying capacity, it is necessary to determine the feeding physiology and the energy budget of the scallops in the aquaculture ecosystem. Furthermore, feeding physiological indices such as filtration rate and absorption efficiency are fundamental parameters in the bioenergetic studies of suspension feeding bivalves (Riisgård 1991). There are studies on the reproduction, spat collection, growth, and aquaculture of this scallop species (Zhang et al. 1956, Wang et al. 1987, Zhang 1992), but little is known about its feeding ecology and physiology.

The ecological and physiological aspects of feeding in bivalve molluscs have long been studied. However, many previous experiments were carried out in the laboratory at various artificially controlled conditions. These laboratory studies on growth and energetics in suspension feeding bivalves achieved peak growth rates that are usually less than the maximal growth rates observed in nature (Kjørboe et al. 1981, Jørgensen 1990). This is because suspension feeding bivalves are exposed to a food supply that consists of a complex mixture of organic and inorganic particles and that fluctuates unpredictably both in quantity and quality in the field, and it has been known that both food quality and quantity are important factors mediating the feeding behavior and physiology of suspension feeders (Bayne and Hawkins 1992). Besides food condition, many other physical and chemical factors such as tem-

perature, salinity, and water flow can also affect the feeding of bivalves. However, it is very difficult to mimic the natural food regimen as well as the above-mentioned physical and chemical conditions in the laboratory. Growth rates comparable with those measured in the field may only be obtained in laboratory experiments that are carried out under simulated natural conditions.

Moreover, there have always been technical and conceptual difficulties in predicting an organism's response in the natural environment from data measured in the laboratory (MacDonald and Ward 1994). This largely restricted the application of laboratory data. Despite these differences, few workers have conducted studies on the feeding strategies of bivalves using natural seston and under simulated natural conditions, and even fewer have evaluated feeding activity when food and other conditions such as temperature varies temporally and seasonally (MacDonald and Ward 1994). However, information regarding changes in feeding behavior under natural conditions is critical to the analysis of bivalve energetics (Bayne et al. 1988). Only those data measured in situ or in the laboratory under simulated natural conditions can be used to readily predict the organism's feeding pattern in nature.

Sungan Bay is located at 37°01'–09'N and 122°24'–35'E (Fig. 1) and is one of the most intensive aquaculture areas in northern China. The main culture species in this bay are scallops, *C. farreri*, and kelp, *Laminaria japonica*. The quantity of cultivated scallops was 2 billion individuals in 1994. At such high densities, scallops may deplete the seston from the water column. It is thus critical to measure the filtration rate and absorption efficiency of scallops in this bay.

In this study, the filtration rate and absorption efficiency of the scallop *C. farreri* were measured in a novel semi-in situ running seawater system using natural seawater (Fig. 2). This is the first time that the filtration rate and absorption efficiency of *C. farreri* were measured. Further, the experiments were carried out four times at the same site in spring and autumn, in order to understand the scallops' feeding regimens under different water conditions. The goal of this research was to understand the cultivated scallops'

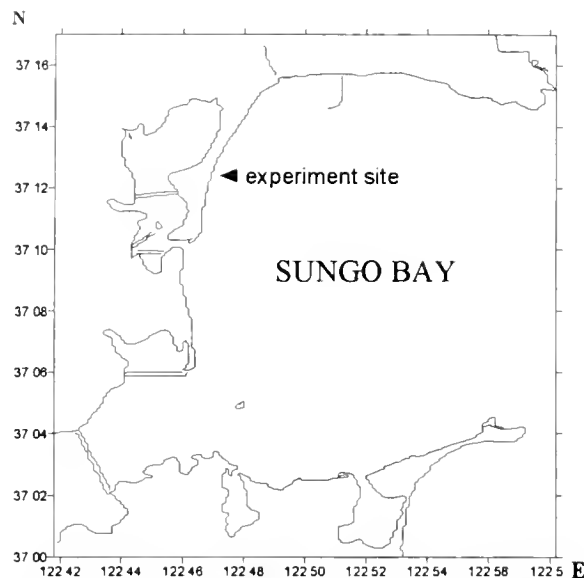


Figure 1. Sungo Bay and the experimental site for measuring filtration rate and absorption efficiency of the scallop *C. farreri*.

feeding strategies in natural conditions by determining the feeding parameters of this species in a simulated natural environment.

MATERIALS AND METHODS

Seawater and Experimental System

Seawater was pumped 50 m from the nearshore off-bottom in Sungo Bay into two large precipitating tanks (500 m³). After precipitating for about 24 h (because of the stirring of the pump, it is better to precipitate the seawater for a period of time to match the seston concentration in nature), seawater was siphoned via a rubber hose with a diameter of 2 cm into a fiberglass tank (0.4 m³) in the hatchery room. The inflowing end of the hose was masked by a screen with pore diameter of 1 mm to prevent large-sized particles from entering the experimental flumes. The fiberglass tank acted as the header tank for the running seawater system in order to maintain a constant water level and flow. Seawater was then siphoned from the fiberglass tank via a 1.5-cm-diameter plastic tube with a multipipe joint at the outflowing end, by which seawater flowed at a controlled and constant flow rate into the experimental flume tanks (Fig. 2). The flume tanks were made of perspex and measured 20 × 20 × 60 cm in length, width, and height. The inflowing hole was 1 cm above the bottom of one end, and the outflowing hole was 1 cm under the surface of the other end (Fig. 2). No extra unicellular algae or seston was added to the

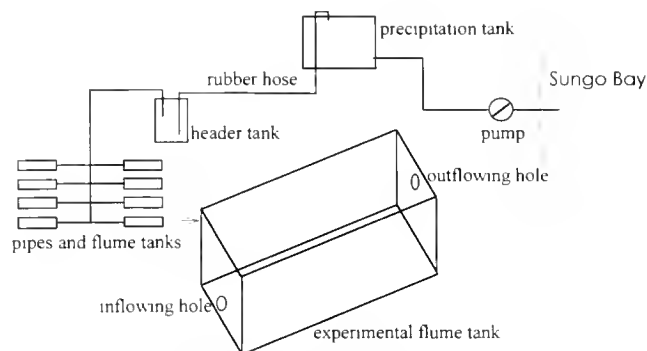


Figure 2. Running seawater system for the measurement of filtration rate and absorption efficiency of *C. farreri*.

experimental seawater. Table 1 lists the experimental seawater conditions at the different seasons.

Scallops

Experimental scallops were collected from a scallop aquaculture site in Sungo Bay, city of Rongcheng, province of Shandong. These randomly selected scallops of different sizes were then carried to the hatchery room of Aitou Farm for the experiments. After the epibiota were cleaned from the shell surface, scallops were grouped according to their size and placed in the running seawater system to acclimate for at least 2 days. Scallops collected in September and November 1993 were divided into six groups, as well as one to two control groups (Table 2). The six experimental groups were marked as S2, M2, B2, S4, M4, and B4; the capital letters S, M, and B represented small-, middle-, and big-sized scallops, and the numbers 2 and 4 indicated the number of individuals in each experimental flume tank. M4, for example, means that there were four middle-sized scallops in this group. The group of scallops measured in May 1994 were divided into three groups marked as S4, M4, and B4. Scallops measured in April 1995 were divided into seven size groups with four individuals per flume tank (Table 2). There were no scallops in the control tanks, but seawater ran through these tanks as in the experimental groups. After the completion of each set of experiments, the shell height and dry tissue weight (60°C for 24 h) of each scallop were recorded. The physical measurements of experimental scallops in different seasons are listed in Table 2.

Experiment Procedures

After 2–5 days of acclimation, individual experimental flume tanks were cleaned of feces and other sediments. A 1,000-mL sample of outflowing seawater from the control and each experimental flume tanks as well as from the fiberglass tank was then

TABLE 1.
Experimental seawater conditions in different seasons.

Dates	Temperature Range (°C)	pH	Salinity (‰)	F (mL/min)	POM (mg/L)	TPM (mg/L)	POM/TPM	PCC (µg/L)
Sept. 9–18, 1993	24.5 ± 0.8	8.12	31.2	520–630	4.40	10.61	0.415	1.98
Nov. 15–24, 1993	10.1 ± 0.5	8.05	32.0	405–522	4.21	9.28	0.454	4.89
May 23–28, 1994	17.9 ± 1.1	8.12	32.1	470–700	3.87	17.66	0.219	3.52
Apr. 21–29, 1995	13.7 ± 1.1	8.02	31.6	309–489	1.09	3.79	0.288	4.45

Abbreviations: F, flow rate at different experimental flumes; PCC, particulate concentration of chl *a*.

TABLE 2.

Characteristics of experimental scallops in different seasons during September 1993 and April 1995.

Date	Group	SH (cm)	DTW (g)	TI* (g/cm)	FR (L/h)	RE (%)	AE (%)
Sept. 1993	S2	3.28 ± 0.19	0.17 ± 0.03	0.052	3.90 ± 0.60	24.35 ± 8.05	43.91
	S4	3.13 ± 0.18	0.17 ± 0.02	0.054	1.91 ± 0.14	21.70 ± 10.11	49.89
	M2	4.15 ± 0.05	0.48 ± 0.08	0.116	5.70 ± 0.13	38.00 ± 18.25	52.60
	M4	3.95 ± 0.07	0.28 ± 0.06	0.071	2.74 ± 0.18	36.35 ± 11.55	35.23
	B2	5.55 ± 0.05	1.20 ± 0.13	0.216	6.17 ± 0.05	38.92 ± 18.66	43.90
	B4	5.64 ± 0.11	1.11 ± 0.09	0.197	4.45 ± 0.93	52.93 ± 21.32	42.03
Nov. 1993	S2	3.03 ± 0.15	0.11 ± 0.02	0.036	0.43	4.33	73.39
	S4	3.45 ± 0.10	0.16 ± 0.06	0.046	0.47	6.21	60.26
	M2	5.05 ± 0.15	0.56 ± 0.08	0.102	0.68	4.98	62.11
	M4	5.05 ± 0.34	0.54 ± 0.11	0.107	0.76	12.53	64.79
	B2	6.90 ± 0.10	1.37 ± 0.13	0.199	2.76	17.64	58.86
	B4	6.65 ± 0.60	1.43 ± 0.26	0.215	1.83	29.26	59.01
May 1994	S4	3.58 ± 0.15	0.17 ± 0.03	0.047	2.10 ± 0.37	27.60 ± 1.95	
	M4	5.13 ± 0.17	0.72 ± 0.11	0.140	2.90 ± 0.43	33.31 ± 4.56	
	B4	6.28 ± 0.29	1.77 ± 0.23	0.282	4.65 ± 1.77	42.87 ± 16.00	
	1	3.05 ± 0.19	0.16 ± 0.02	0.052	1.35 ± 0.77	22.51 ± 18.05	61.60
Apr. 1995	2	3.65 ± 0.06	0.27 ± 0.03	0.074	1.84 ± 0.86	33.77 ± 9.72	62.27
	3	4.10 ± 0.26	0.41 ± 0.01	0.100	2.17 ± 1.61	32.84 ± 19.95	56.64
	4	4.48 ± 0.21	0.47 ± 0.08	0.105	2.44 ± 0.79	41.71 ± 6.16	60.59
	5	5.38 ± 0.05	1.01 ± 0.10	0.188	2.58 ± 1.26	47.00 ± 14.29	62.80
	6	5.73 ± 0.05	1.16 ± 0.14	0.202	2.95 ± 1.48	46.39 ± 20.85	60.26
	7	6.63 ± 0.06	1.44 ± 0.03	0.217	3.31 ± 1.36	47.82 ± 14.63	60.72

Abbreviations: SH, shell height; DTW, dry tissue weight; TI, tissue index; FR, filtration rate; RE, retention efficiency; AE, absorption efficiency.

*TI = DTW/SH × 100.

collected every 6 h—500 mL for analysis of chlorophyll *a* (chl *a*) concentration, and another 500 mL for analysis of particle organic matter (POM) and total particle matter (TPM). Each experiment lasted for 24 h; thus, we sampled five times for each experiment. Water flow rates were calculated from the volume of collected seawater over a known time period. Preliminary analysis of filtration rates determined by the differences of chl *a* and POM in the inflowing and outflowing seawater, respectively, indicated that filtration rate determined by chl *a* (difference) was more stable and reliable than that determined by POM (Kuang et al. 1996a). Therefore, differences of chl *a* concentrations between the inflowing and outflowing seawater were only used to calculate the filtration rate in the following experiments, whereas differences of POM/TPM between the seawater and feces were used to calculate the absorption efficiency. After precipitating for 24 h, the seston concentrations in the fiberglass tank and in the outflowing water of the control group were comparable, and the seston concentration in the outflowing seawater of the control tanks was used to estimate the seston supply in the experimental groups. Feces were collected gently with a pipette at the end of each experiment (24-h period). Because of electrical power failure, the feces collected in May 1994 cannot be processed; thus, the related absorption efficiencies were omitted.

Analysis of Chl *a*

The concentrations of chl *a* were determined according to Parsons et al. (1992). The 500-mL seawater samples were filtered through acetate fiber membranes (pore size, 0.45 µm) under 0.5-MPa atmospheric pressure vacuum. As the seawater was being filtered, a few drops of a suspension of magnesium carbonate (MgCO₃) were added to prevent acidity on the filter. After filtration, filters were placed in 10-mL centrifuge tubes and chl *a* was

extracted in 90% acetone overnight in a cold (4°C), dark place. The contents of each tube were centrifuged for 10 min at 4,000 revolutions/min after extraction. The extinctions of the supernatants were then measured immediately at 750-, 664-, 647-, and 630-nm wavelength using a 2-cm path length model 7230 spectrophotometer. The chl *a* was calculated as follows:

$$\text{chl } a \text{ (}\mu\text{g/L)} = (11.85E_{664} - 1.54E_{647} - 0.08E_{630} - 10.23E_{750}) \times v/(V \times l),$$

where E_{664} , E_{647} , E_{630} , and E_{750} are the extinctions at 750-, 664-, 647-, and 630-nm wavelength, respectively; v is the volume (mL) of acetone; V is the volume (L) of the seawater sample; l is the path length (cm) of the spectrophotometer.

Analysis of POM and TPM

The 500-mL seawater samples were filtered onto preashed and preweighed (W_0) 40-mm GF/C-grade filters (nominal pore size, 1.2 µm) under a 0.03-MPa vacuum. Approximately 10 mL of distilled water was added to the last few milliliters of the sample to remove residual salts. Filters with seston were then frozen at -20°C in a desiccator in the dark until they were transported back to the laboratory for processing. The TPM concentrations were determined after drying filters at 60°C for 48 h and weighting (W_{60}) (TPM = $W_{60} - W_0$). These filters were then ashed in a muffle furnace at 450°C for 5 h and weighted (W_{450}). POM contents were calculated as follows: POM = $W_{60} - W_{450}$. A Sartorius Research R 200D electronic semimicrobalance was used for weighting. The total weight and organic portion of feces were determined by the same method as seawater samples above.

Calculations

Filtration rate (FR), also termed as clearance rate (CR), was measured as the volume of water cleared of chl *a* per unit time; it

was calculated as follows: $FR = F \times (C_1 - C_2)/C_1$, where F is the flow rate of water through the experimental flumes (L/h), C_1 is the chl a concentration in the inflowing seawater, and C_2 is the chl a concentration in the outflowing seawater after it has been processed by the scallops. FR values presented in this study were the mean values per individual.

Retention efficiency (RE) was calculated as follows: $RE = (1 - C_2/C_1) \times 100$. RE values presented in this study were the group values. Absorption efficiency (AE) for ingested food is estimated by measuring the dry weight (DW , = TPM) and ash-free dry weight ($AFDW$, = POM) of seston in inflowing seawater and fecal pellets from scallops. The POM/TPM of food and feces was then used to calculate the absorption efficiency: $AE = (1 - E/F)/(1 - E) \times 100$ (Conover 1966a), where F = POM/TPM of inflowing seawater and E = POM/TPM of scallop feces. Data analyses and statistical tests were performed with Microsoft EXCEL 5.0.

RESULTS

Summary of Experimental Conditions

Table 1 reported the environment conditions of experimental seawater, and Table 2 reported the shell height, body weight, and tissue index of scallops. Data in Table 1 represent the natural seawater conditions in the experimental site and were comparable to the results of a synchronous biological and hydrochemical survey of the bay (Kuang et al. 1996b). Tissue indices of scallops varied with body size and season. This was related to the scallop's gametogenesis and reproductive cycle (Zhang et al. 1956). *C. farreri* has two reproductive peaks per year; the minor one occurs during middle May and early June, and the major one occurs in October (Zhang et al. 1956). In our study, the scallops' highest tissue indices occurred in September, followed by May, April, and November (Fig. 3). This is because scallops in September have not yet spawned, so they have the highest tissue indices, whereas scallops in November were spent, so they have the lowest tissue indices.

Filtration Rate

Filtration rates of scallops are listed in Table 2. Data analysis indicated that the filtration rate of scallops increased with size

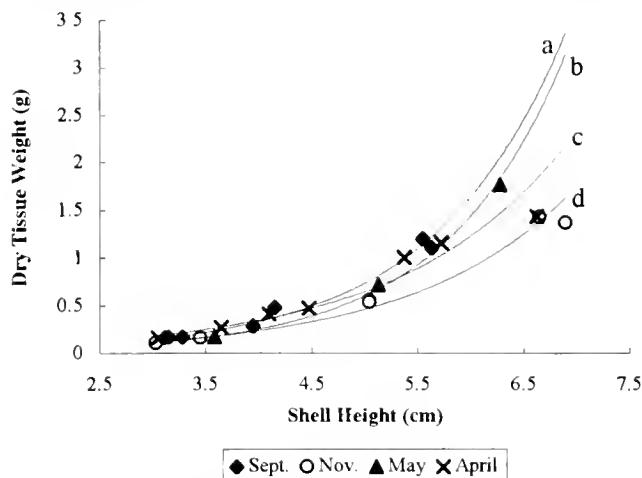


Figure 3. Dry tissue weight (W) of the scallop *C. farreri* as a function of shell height (H) in different seasons. Regression equations for different seasons are as follows: a (Sept.): $W = 0.0134e^{0.80H}$, $R^2 = 0.974$; b (May): $W = 0.0077e^{0.87H}$, $R^2 = 0.998$; c (Apr.): $W = 0.027e^{0.64H}$, $R^2 = 0.962$; d (Nov.): $W = 0.0164e^{0.67H}$, $R^2 = 0.984$.

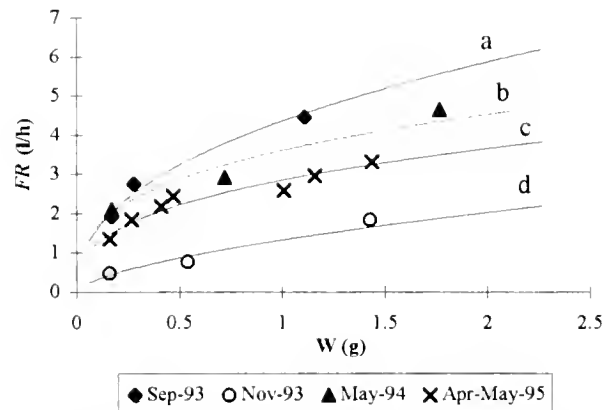


Figure 4. Filtration rate (FR) of the scallop *C. farreri* as a function of dry tissue weight (W) in different seasons. Regression equations for different seasons are as follows: a (Sept.): $FR = 4.35W^{0.43}$, $R^2 = 0.97$; b (May): $FR = 3.60W^{0.33}$, $R^2 = 0.94$; c (Apr.): $FR = 2.85W^{0.36}$, $R^2 = 0.93$; d (Nov.): $FR = 1.33W^{0.61}$, $R^2 = 0.95$.

(weight), and the relationship between these two variables can be represented as $FR = aW^b$. Our results showed that the exponent b of filtration rate as a function of dry tissue weight in different seasons ranged from 0.33 to 0.61 with an average of 0.43, whereas the elevations a varied more widely between 1.33 and 4.35 (Fig. 4). Figure 4 showed that filtration rate varied among seasons and decreased in the order of September, May, April, and November; this was consistent with the order of seawater temperature. Analysis showed that filtration rate was correlated with the seawater temperature—the higher the seawater temperature, the higher the scallop's filtration rate (Fig. 5). Results in September and November 1993 showed that the filtration rate of scallops was also related to their densities—the higher the scallop densities, the lower the individual filtration rate (Table 2). Although seston quantity and quality (POM ratio in TPM) also varied among seasons, they did not seem to be the dominant factors that directly influenced the filtration rate of scallops in natural seawater conditions (Table 1).

Retention Efficiency

Similar to filtration rate, retention efficiency of the different groups increased in relation to body size (Table 2) and dry tissue weight (Fig. 6). The highest retention efficiency occurred in September, followed by April and May, whereas the lowest value occurred in November (Fig. 6). Unlike the filtration rate, both the exponent (range, 0.18–0.70; average, 0.41) and the elevation (range, 21.47–52.98; average, 39.37) of retention efficiency varied widely as a function of dry tissue weight among the different seasons.

Absorption Efficiency

Unlike the filtration rate and retention efficiency, the absorption efficiency of the scallops had no relationship to body size/weight, and the absorption efficiency values of scallops of different body sizes varied in a narrow range in the same month (Table 2; also see SD of absorption efficiency below). However, scallops in different months had different mean absorption efficiency values. Although scallop absorption efficiencies in November (average \pm SD, 63.07 \pm 5.52%) and April (average \pm SD, 60.70 \pm 2.02%) were not significantly different (analysis of variance [ANOVA], $F = 1.12$, $df = 12$, $p = 0.31$), the absorption effi-

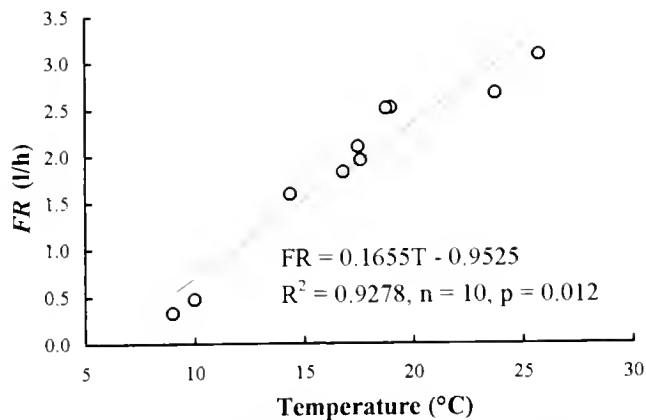


Figure 5. Filtration rate (FR) of *C. farreri* as a function of seawater temperature (T). Data are not based on the same batch of scallops, but on different scallops of the same dry tissue weight (0.16–0.17 g/individual) in different seasons.

ciencies of these two months were higher than that of September (average \pm SD, $44.59 \pm 6.12\%$) (ANOVA, between September and November, $F = 30.14$, $df = 11$, $p = 0.00026$; between September and April, $F = 43.56$, $df = 12$, $p = 0.00004$). It seemed that the higher the chl *a* concentration (seston quality) and the more suitable the environmental condition (temperature), the higher the scallop's absorption efficiency. In this case, the food quality (higher chl *a* concentration; Table 1) and environmental conditions (e.g., temperature) in November and April resulted in higher absorption efficiency for this species, compared with September, which had high seawater temperatures and low chl *a* concentrations (Tables 1 and 2).

DISCUSSION

Filtration rate is a dynamic index that reflects the physiological feeding state of suspension feeding animals. It is reported that temperature (e.g., Newell et al. 1977, Buxton et al. 1981), salinity (e.g., Loosanoff 1953, Navarro 1988), variation in food quantity and quality (e.g., Higgins 1980a, Higgins 1980b, Riisgård 1988, Riisgård 1991, MacDonald and Ward 1994), and other environmental parameters (e.g., Walne 1972, Shumway et al. 1983, Bricelj

and Malouf 1984, Bayne et al. 1987, Cranford and Grant 1990) can all influence the filtration rate of bivalves. It seemed that the seasonal variation in the filtration rate of the scallop *C. farreri* was mainly decided by seawater temperature. Our results show that the changes of the scallop's filtration rate in different seasons were consistent with the changes of natural seawater temperature. This trend was comparable to that of the sea scallop *Placopecten magellanicus*, measured by MacDonald and Thompson (1986) under ambient temperatures and natural seston levels. However, Thompson (1984) reported that there were no seasonal patterns in clearance rate for the mussel *Mytilus edulis*. The filtration rate of *C. farreri* reported in this article is lower than that of the Pacific oyster *Crassostrea gigas*, measured at the same site and at the same time (Kuang et al. 1996d). Although food quantity (TPM content) and quality (POM percent in TPM, or chl *a* content) may affect the filtration rate of *C. farreri* in single-factor experiments, they did not seem to be major factors influencing the scallop's seasonal filtration rate patterns in the natural environment. For example, the highest TPM content occurred in May and the highest percent POM occurred in November, but the highest filtration rate was in September. Water flow in this experiment also did not affect filtration rate, even though our experimental seawater flows were faster than those used in previous studies (e.g., MacDonald and Ward 1994). *C. farreri* was most often found under conditions of fast flow rates in nature (Zhang et al. 1962). In this study, for example, scallops often assembled themselves near the inflowing hole in the experimental flume tank, where the water flow was relatively faster. Other studies have reported that the filtration rate of *C. farreri* remained constant in the water flow range of 300–600 mL/min (Kuang et al. 1996c). Hildreth (1976) reported that the filtration rate of blue mussel was unresponsive to changes in flow rate of 2–41 L/h. Therefore, in *C. farreri*, the seasonal filtration rate pattern was related more to temperature than to food or other aspects of the seawater.

An allometric relationship of group retention efficiency as a function of dry tissue weight was observed in this study. The retention efficiency of bivalves was reported, by many authors, to vary with particle sizes (e.g., Shumway et al. 1983, Cranford and Grant 1990, MacDonald and Ward 1994). It is possible that the seasonal patterns of retention efficiency in *C. farreri* may be affected by several factors.

The measurement of absorption efficiency in this article indicated that there were no differences among individuals of different sizes, but that there were differences between seasons. It has long been believed that food quality, rather than temperature, food quantity, or other variables, is the major factor affecting the absorption efficiency (Conover 1966b, Vahl 1980, Bayne et al. 1988, Navarro et al. 1991, Navarro et al. 1992, Iglesias et al. 1992, Navarro and Iglesias 1993, Cranford 1995). Cranford (1995) reported that diet quality, expressed as POM, POC (particle organic carbon), or PN (particle nitrogen) content per unit of the particulate matter, explained between 74 and 84% of the variance in sea scallop absorption efficiency measurements. Our results suggest that food quality (chl *a* concentration) may explain the variation of absorption efficiency between different months. The seasonal absorption efficiency patterns may also be related to several other factors. Despite the higher POM proportion in September, the low chl *a* concentration and less "comfortable" environmental condition (e.g., elevated seawater temperature—the most suitable temperature for the growth of *C. farreri* is 15–20°C) resulted in a lowered absorption efficiency. The absorption efficiencies mea-

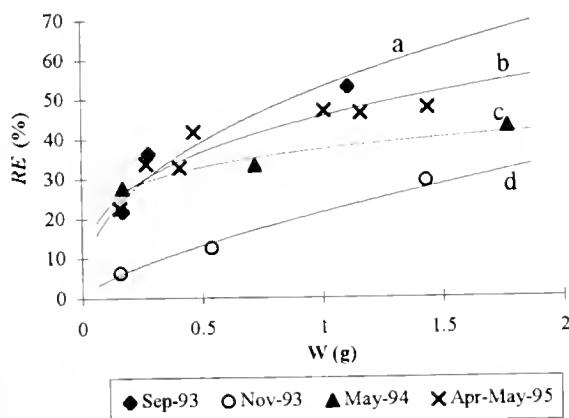


Figure 6. Retention efficiency (RE) of the scallop *C. farreri* as a function of dry tissue weight (W) in different seasons. Regression equations for different seasons are as follows. a (Sept.): $RE = 52.98W^{0.43}$, $R^2 = 0.88$; b (Apr.): $RE = 45.69W^{0.31}$, $R^2 = 0.87$; c (May): $RE = 37.35W^{0.18}$, $R^2 = 0.95$; d (Nov.): $RE = 21.46W^{0.70}$, $R^2 = 0.98$.

sured in this study were within the range of other bivalves; Powell and Stanton (1985) found an average absorption efficiency of 0.54 for bivalves. However, compared with that of the Pacific oyster *C. gigas* (Kuang et al. 1996d), the absorption efficiency of *C. farreri* was relatively low. The average absorption efficiency of the Pacific oyster in the same environmental conditions is 80%. Oysters can increase their absorption efficiency by selective ingestion and then produce a large amount of pseudofeces. However, the scallop *C. farreri* produced very few pseudofeces during our experiment, although they sometimes produced large amounts of pseudofeces during the artificial cultivation of broodstocking when food concentration was very high. Similar results also have been reported for *P. magellanicus*, *M. edulis*, and *Cardium edule* when feeding on natural seston (MacDonald and Thompson 1986, Newell and Bayne 1980). In this study, scallops were exposed to natural seston and ambient temperatures, and the results may be more environmentally representative.

The exponents of the allometric equation relating filtration rate to dry tissue weight varied in a narrower range than that of retention efficiency (Figs. 4 and 6). However, the averaged exponents of 0.43 for the filtration rate and 0.41 for the retention efficiency were very similar. These slopes were relatively low compared with those of other bivalve species. The common exponents of 0.70 for *P. magellanicus*, 0.60 for *Chlamys islandica* (MacDonald and Thompson 1986), 0.58 for *Argopecten irradians* (Kirby-Smith 1972), 0.82 for *Pecten irradians* (Chipman and Hopkins 1954), and 0.66 for *M. edulis* (Mohlenberg and Riisgård 1979), and an overall range of 0.4–0.6 for bivalves (Officer et al. 1982, Winter 1978), have been reported.

Many previous studies have determined filtration rates and absorption efficiency of bivalves under conditions of one individual per experimental chamber (e.g., MacDonald and Thompson 1986). Our experiments, however, have been adopted a typical density of four individuals per flume tank. Theoretically speaking, this may underestimate the results because seston may be depleted by scallops in high density. However, the purpose of this study is to predict the feeding regimens of *C. farreri* cultivated in Sungo Bay, and the densities of scallop-intensive culture in the bay were very high (50 individuals/m²). The densities adopted by this study are comparable to the scallop aquaculture density in the bay. Furthermore, water flow rate in this study was higher than in previous studies, and the volume of the experimental flume tank is large enough to accommodate four scallops; in such conditions, it is not possible for the scallops to deplete the seston. Although the seasonal patterns of the filtration and absorption in *C. farreri* have been recorded, the mechanisms that scallops use to regulate their feeding physiological change and the feeding physiology of larval *C. farreri* have yet to be determined.

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CLEARANCE AND INGESTION RATES OF *ISOCHRYYSIS GALBANA* BY LARVAL AND JUVENILE BAY SCALLOPS, *ARGOPECTEN IRRADIANS CONCENTRICUS* (SAY)

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ABSTRACT The clearance and ingestion rates of larval and juvenile bay scallops *Argopecten irradians concentricus* were investigated using algal suspensions of *Isochrysis galbana*. An inverse relationship existed between clearance rate and algal cell concentration. Mean clearance rate ranged from 0.0034 to 0.0385 mL h⁻¹ for larvae and 0.14 to 0.41 mL h⁻¹ for juveniles of 0.5 mm shell height and increased to 248–420 mL h⁻¹ for juveniles of 10 mm shell height. Clearance rate increased with shell size allometrically, with a mean exponent of 2.460 ± 0.087 . Weight-specific clearance rate was independent of shell size at >20 cells μL^{-1} , but slightly decreased with increasing shell size at lower cell concentrations. Larvae and juveniles showed higher ingestion rates at higher algal concentrations, and the hyperbolic relationship was described using an enzymatic kinetic equation. Maximum ingestion rate occurred at approximately 20 cells μL^{-1} in larvae and at >20 cells μL^{-1} in juveniles. As temperature increased from 10 to 30°C, larvae and juveniles became more active in feeding. Relative rates of clearance and ingestion at 10, 15, 20, and 25°C were 8.4, 22.0, 52.8, and 88.2%, respectively, of the mean rate at 30°C.

KEY WORDS: Bay scallops, *Argopecten irradians*, feeding, food uptake, clearance, ingestion

INTRODUCTION

The growth of larval and juvenile bay scallops over several developmental stages at various *Isochrysis galbana* concentrations has been reported (Lu and Blake 1996). Larval and juvenile bay scallops have been shown to reach optimal growth at 10–20 cells μL^{-1} of *I. galbana*. Although growth continued to increase at higher *I. galbana* concentrations, the increase was statistically insignificant. A complete understanding of the interaction between growth and food supply needs the knowledge of feeding response of those stages to changes in food supplies.

The ability of bivalves to maintain a positive energy balance depends primarily on food ingestion. Therefore, a great deal of work has been done on feedings of adult bivalves, including the bay scallop. Previous studies have shown that the clearance rate of the bay scallop was related to shell height following a general allometric equation (Chipman and Hopkins 1954, calculated by Winter 1978, Kirby-Smith 1970), but it was inversely related to cell concentration (Palmer 1980, Kuenstner 1988).

Very limited data are available, however, on the feeding physiology of larval and juvenile bay scallops. The only data available on the feeding of larval bay scallops appear to be that of Gallagher et al. (1989). However, extrapolation of their results is difficult because they determined ingestion and clearance at only one cell concentration each of *I. galbana* and *Aureococcus anophagefferens*. Feedings of several size groups of juvenile bay scallops were studied using cultured algae and natural assemblages of particular organic matter (Bricelj and Kuenstner 1989, Cahalan et al. 1989, Shumway et al. 1996), but a clear relationship between feeding rate and body size is still not available.

In this study, clearance and ingestion rates of larval and juvenile bay scallops, *Argopecten irradians concentricus* (Say), were determined. To investigate the effect of body size and cell concentration on feeding activity, three larval classes and seven juvenile classes (0.5–10 mm in shell height) were tested at six cell concentrations of *I. galbana*. The effect of temperature on clearance rate and ingestion rate was determined using juveniles approximately 5 mm in shell height.

MATERIALS AND METHODS

Adult bay scallops were collected from Homosassa, FL, and experiments were carried out at the Department of Marine Science, University of South Florida at St. Petersburg, FL. Ripe scallops were allowed to spawn in seawater of 25–28‰ salinity at 24–26°C. Fertilized eggs were allowed to develop for 20–30 h until they developed to D-shaped larvae. Larvae were collected using a 35- μm screen and were transferred to 500-L stock tanks. They were cultured at a density of 4–8 mL⁻¹ and fed twice daily in the total amount of 10–30 cells μL^{-1} of *I. galbana*, which was grown in f/2 media (Guillard and Ryther 1975). At the end of the planktonic stage, black plastic *Thalassia* mimics were added to the larval tank as substrate for larvae settlement. The daily food ration for juveniles was increased gradually from 30 to 100 cells μL^{-1} of *I. galbana*.

Larvae used for feeding experiments were collected from the 500-L stock tanks. They were not fed 12 h before the start of each experiment. Larvae were filtered onto a 35- μm screen, washed with filtered seawater (Whatman G4, 1.2 μm), and released into 1,000 mL of filtered seawater. Larval density was determined by counting five 2-mL samples. Aliquots of the larval stock were placed in 1-L glass beakers, and filtered seawater was added to a final volume of 500 mL. Larval density was maintained at about 3–10 mL⁻¹. Clearance rate was determined at 25°C under six *I. galbana* concentrations (1–60 cells μL^{-1}) for 4–6 h. All experimental cultures were duplicated. A set of beakers with no larvae were used as controls to determine the changes of cell concentration unrelated to larval feeding, such as cell division and cell sinking. Gentle aeration was supplied to each test beaker to keep the algae in suspension. Samples of 20 mL were drawn from each beaker with a pipette at the beginning of the experiment and every 60 min thereafter. Samples were passed through a 35- μm screen to remove larvae. *I. galbana* cells were counted twice for each sample with a Coulter Counter Model FN fitted with a 100- μm aperture tube.

Clearance rate (CR) (modified from Coughlan 1969) and weight-specific clearance rate (CR_w) were calculated using the following equations:

$$CR = [\ln(C_i/C_f)]/(td)$$

$$CR_s = CR/AFDW$$

where C_i and C_f are the initial and final cell concentrations; t is the time interval; d is the density of larvae; and AFDW is ash free dry weight of larvae or juveniles (Lu and Blake 1996). At any cell concentration (C), ingestion rate (IR) was obtained by the equation:

$$IR = CR \times C$$

Similar experimental procedures were used to determine clearance and ingestion rates for juveniles (0.5–10 mm shell height), except that juveniles were kept at lower densities to avoid a sharp reduction of cell concentrations in the experimental beakers by feeding. Shell height of juvenile scallops was measured under a compound microscope fitted with a micrometer. Only those within $\pm 5\%$ of the proposed shell size were picked out for each study. Juvenile densities used were adjusted according to size, ranging from one 0.5-mm juvenile per 10 mL of medium to one 10-mm juvenile per 2,000 mL of medium. Experiments lasted 3–5 h.

Juveniles of approximately 5 mm shell height were used to determine the effect of temperature on the feeding activity of juvenile bay scallops. Experimental temperatures ranged from 10 to 30°C at 5°C intervals. Before each measurement, juveniles were kept at $\pm 0.5^\circ\text{C}$ of the experimental temperature for 48 h. Two juveniles were placed in each of the 1-L beakers containing 600 mL of experimental medium. All feeding regimens were duplicated. Six beakers (one for each cell concentration) with no juveniles were set up as controls.

RESULTS

Measured clearance rates were plotted against *I. galbana* (3–6 μm) concentration for each of the 10 size classes of larval and juvenile bay scallops, and results are shown in Figure 1. Clearance rate at each of the six standardized cell concentrations (1, 5, 10, 20, 30, and 50 cells μL^{-1}) was obtained by weight averaging of the measured rates, and results are summarized in Table 1 and illustrated in Figure 1. In all cases, clearance rate was high at low cell concentrations but decreased as cell concentrations increased. There was a 60–90% decline in clearance rate as cell concentration increased from 1 to 50 cells μL^{-1} . Such declines were more obvious for larval and small juvenile classes. At the lowest cell concentration of 1 cell μL^{-1} , a slight decline in clearance rate compared with that at 5 cells μL^{-1} was observed in the 1.1-, 2.1-, and 3.1-mm juvenile classes, but not in the larval classes or the rest of juvenile classes.

Clearance rates increased with increasing larval and juvenile size at all cell concentrations. Allometric equations were fitted to the clearance rate–shell size and clearance rate–AFDW relationships, and the fitted parameters are given in Table 2. Fitted b -values for the clearance rate–shell height relationships were very close to each other and ranged from 2.312 to 2.546 (mean = 2.460). Two representative curves of the relationships are shown in Figure 2. The b -values for the clearance rate–AFDW relationships ranged from 0.868 to 0.956, with a mean of 0.923.

Weight-specific clearance rates were similar throughout the animal size range tested at >20 cells μL^{-1} cell concentrations (Table 1). At cell concentrations <10 cells μL^{-1} , however, there was a general decline in weight-specific clearance rate with increasing shell size.

Cell ingestion rate–body size relationships were converted from the clearance rate–body size relationships. Therefore, ingestion rate showed the same pattern as clearance rate in relation to body size. Both rates had the same b -values at corresponding cell concentrations (Table 2).

Figure 3 shows cell ingestion rate–cell concentration relationships of two size classes of the bay scallop. Ingestion rate increased rapidly with the increase of cell concentration at low concentrations and leveled off at higher concentrations. A 10- to 20-fold increase in ingestion rate was observed over the cell concentration range of 1–50 cells μL^{-1} in all size classes. Ingestion rates of each size class were fitted to an enzymatic kinetic equation:

$$IR = IR_{\max} \times C/(K_s + C)$$

where IR_{\max} is the estimated maximum ingestion rate, K_s is the half-saturation concentration, and C is the cell concentration. Figure 4 shows the two parameters fitted, IR_{\max} and K_s , in relation to juvenile shell height. The relationship of IR_{\max} versus size is exponential, whereas that of K_s versus size is hyperbolic.

Production of pseudofeces was observed at 30 and 50 cells μL^{-1} in the experiment with 2-mm juveniles. However, no pseudofeces were produced in an experiment done a day later using the same juveniles under the same experimental conditions. Pseudofeces were also observed in the experiment with 3-mm juveniles at >10 cells μL^{-1} , but not in the rest of the experiments with juveniles of other size classes.

The highest clearance rates were found at high temperatures and low cell concentrations (Table 3). The data in Table 3 were regressed against temperature and cell concentration, and results were plotted in Figure 5. When clearance rates were expressed as a percentage of maximum rate, a sharp increase in clearance rate with temperature was obvious at all of the six cell concentrations (Fig. 6). Mean relative clearance rates were 88.2, 52.8, 22.0, and 8.4% at temperatures of 25, 20, 15, and 10°C, respectively, with respect to that at 30°C.

Two-way analysis of variance showed that clearance rates and ingestion rates were significantly affected by temperature and cell concentration (Table 4). Multiple range tests demonstrated that clearance rate and ingestion rate were significantly affected by temperature in the range of 15–25°C, whereas they were not significantly different between 10 and 15°C and between 25 and 30°C (Table 5).

Ingestion rates in relation to temperature are shown in Figure 7. Hyperbolic relationships were found between ingestion rates and temperatures between 15 and 30°C. The relationships broke down at temperature below 15°C, where the determined ingestion rates were much higher than that predicted by the hyperbolic curves.

DISCUSSION

Results of this study show that larvae of 150 μm shell length had the highest clearance rate among the three larval classes: 120, 150, and 180 μm . This agrees with results of the growth studies for this species (Lu and Blake 1996), in which it was found that the maximum larval growth occurred at shell lengths of 150–170 μm . In the feeding experiment with the 180- μm class, all of the larvae used had developed eye-spots, a sign indicating that they were ready to settle and metamorphose. This may have led to the slightly lower clearance rates observed for this size class, because

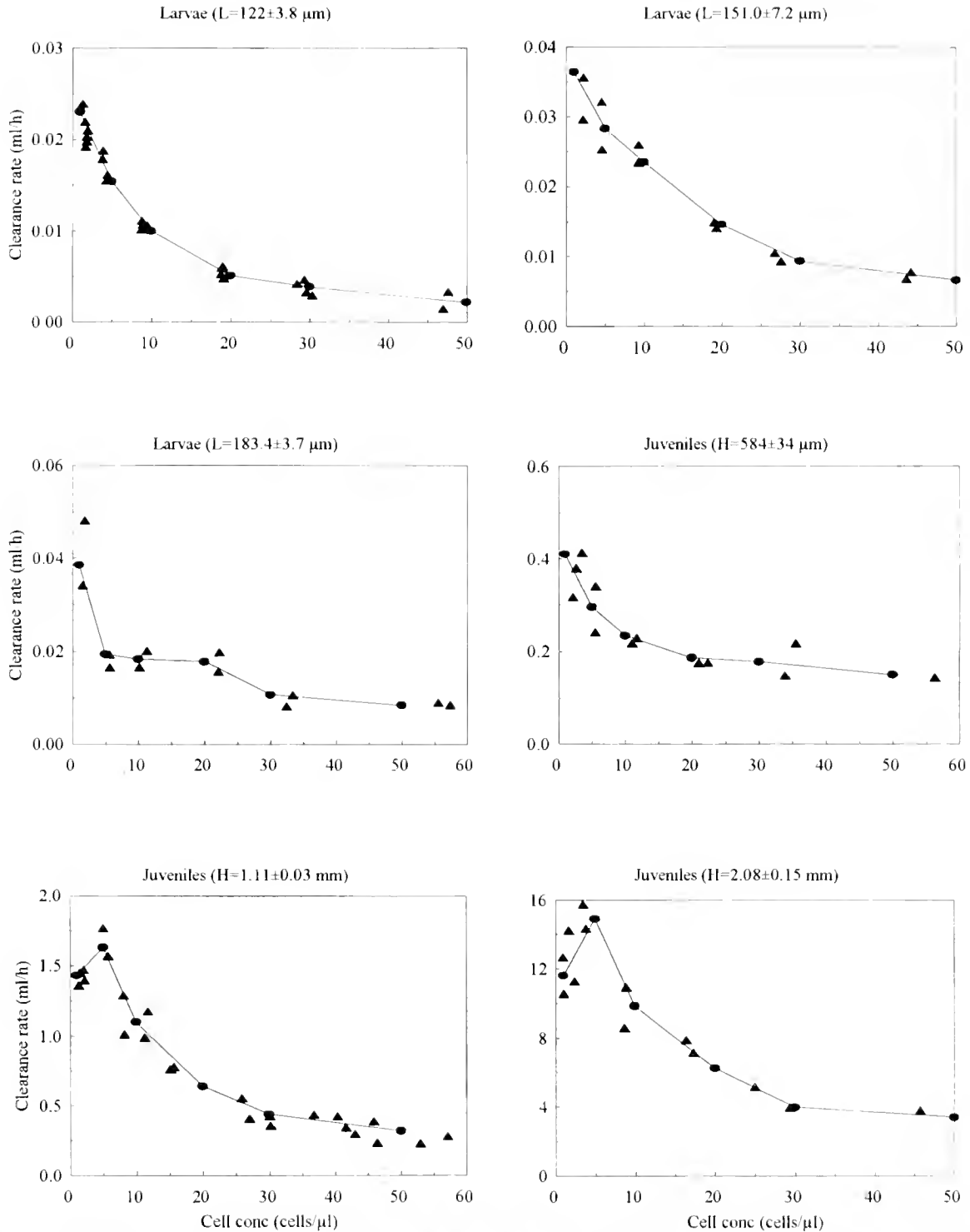


Figure 1. *A. i. concentricus*. Clearance rate of larvae and juveniles of various size classes in relation to *I. galbana* concentration (conc). L., length; H., height.

during metamorphosis, larvae lose their vela and are unable to feed, relying entirely on energy reserves accumulated during their planktonic stage (Yonge 1947, Sastry 1965, Bayne 1965).

Clearance rates of 1.2–8.2 $\mu\text{L h}^{-1}$ were reported for *Argopecten irradians irradians* larvae at 50 cells μL^{-1} of *I. galbana* (Gallager et al. 1989). Those values are similar to the rates of

3.4–7.8 $\mu\text{L h}^{-1}$ determined for *A. i. concentricus* at the same *I. galbana* concentration in this study. Our values (3.4–38.5 $\mu\text{L h}^{-1}$ at 1–50 cells μL^{-1}) for the larvae of *A. i. concentricus* are also comparable to that of other bivalve larvae: 4–52 $\mu\text{L h}^{-1}$ for *Mytilus edulis* (Sprung 1984), about 4–55 $\mu\text{L h}^{-1}$ for *Mercenaria mercenaria* (Riisgård 1988), and about 10–45 $\mu\text{L h}^{-1}$ for *Pati-*

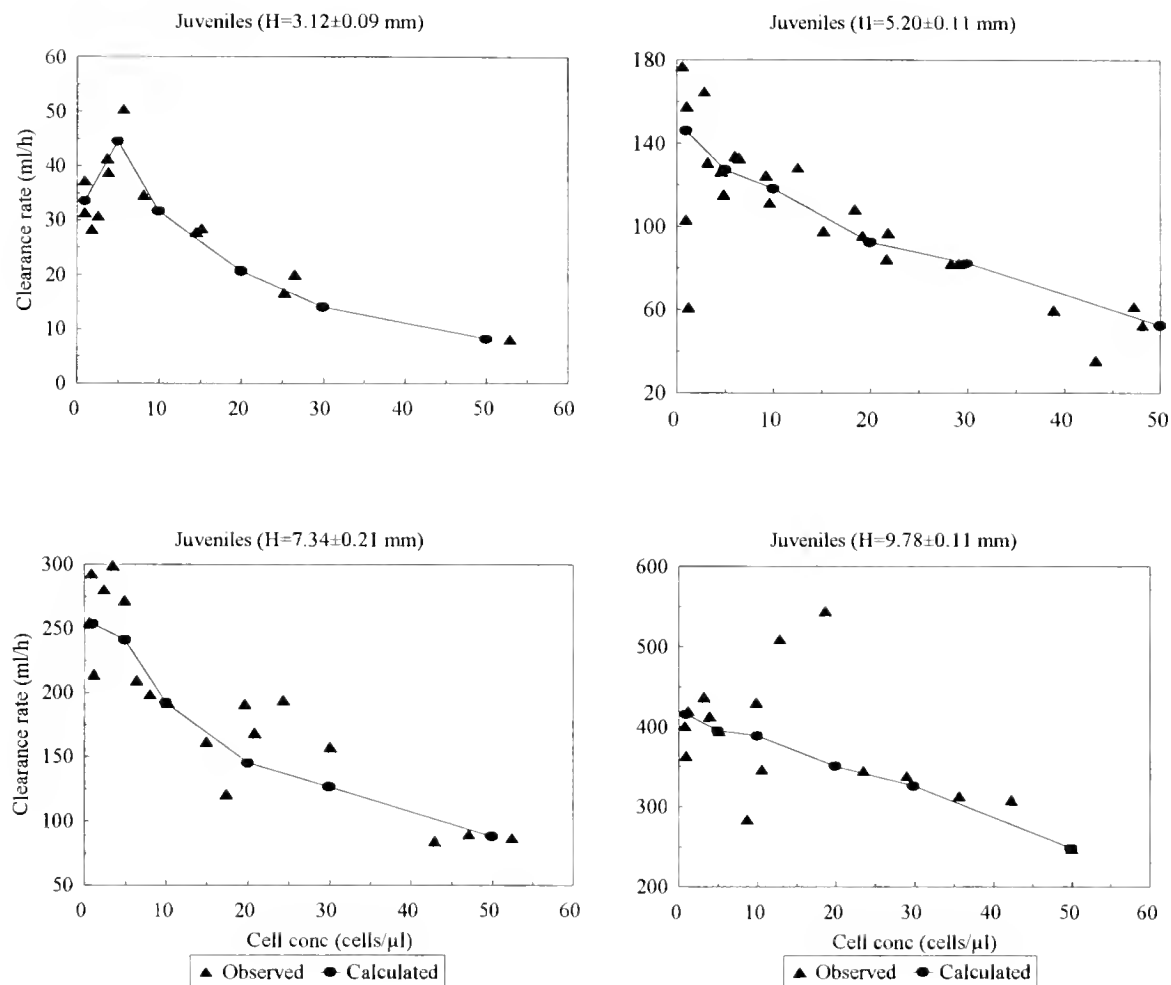


Figure 1. Continued.

nopecten yessoensis (MacDonald 1988) over similar *I. galbana* concentrations. Higher clearance rates of 8.2–106 $\mu\text{L h}^{-1}$ were reported for *M. edulis* in another study (Jespersen and Olsen 1982), and slightly lower rates (2–17 $\mu\text{L h}^{-1}$) were documented for *Ostrea edulis* larvae (Beiras and Camacho 1994). A summary on clearance rates of bivalve larvae can be found in Sprung (1984).

At >20 cells μL^{-1} of *I. galbana*, larvae and juveniles in this study had similar weight-specific clearance rates. At lower cell concentrations, however, larvae had higher weight-specific clearance rates than juveniles, indicating that larvae are more efficient at obtaining food at low cell concentrations than juveniles. Such an adaptation may enable larvae to exploit more efficiently the food

TABLE 1.

A. i. concentricus: clearance rate of larvae and juveniles of various sizes at the six standard cell concentrations ($\text{c}\cdot\mu\text{L}^{-1}$) of *I. galbana* concentrations.

Height (mm)	Clearance Rate (mL ind^{-1} per h)						Weight-Specific Clearance Rate (mL mg^{-1} [AFDW] per h)					
	1 $\text{c}\cdot\mu\text{L}^{-1}$	5 $\text{c}\cdot\mu\text{L}^{-1}$	10 $\text{c}\cdot\mu\text{L}^{-1}$	20 $\text{c}\cdot\mu\text{L}^{-1}$	30 $\text{c}\cdot\mu\text{L}^{-1}$	50 $\text{c}\cdot\mu\text{L}^{-1}$	1 $\text{c}\cdot\mu\text{L}^{-1}$	5 $\text{c}\cdot\mu\text{L}^{-1}$	10 $\text{c}\cdot\mu\text{L}^{-1}$	20 $\text{c}\cdot\mu\text{L}^{-1}$	30 $\text{c}\cdot\mu\text{L}^{-1}$	50 $\text{c}\cdot\mu\text{L}^{-1}$
0.122	0.0230	0.0154	0.0101	0.0051	0.0039	0.0034	266.82	178.65	116.01	59.16	45.24	25.52
0.151	0.0364	0.0284	0.0235	0.0146	0.0093	0.0078	223.47	174.36	144.27	100.68	57.10	40.52
0.183	0.0385	0.0194	0.0183	0.0178	0.0107	0.0075	128.95	64.98	61.29	59.62	35.84	28.47
0.548	0.41	0.30	0.23	0.19	0.18	0.14	147.50	106.49	84.18	66.91	64.04	53.96
1.112	1.43	1.63	1.10	0.64	0.44	0.32	78.10	89.02	59.97	34.95	24.03	17.48
2.080	11.60	14.90	9.85	6.24	3.98	3.40	119.47	153.46	101.45	64.27	40.99	35.02
3.100	33.50	44.50	31.64	20.59	14.00	8.10	119.17	158.30	112.56	69.37	46.25	28.81
5.500	145.96	127.14	118.16	92.38	82.14	52.45	112.73	98.19	91.26	71.35	63.44	40.51
7.300	253.50	241.00	192.10	144.71	126.46	87.90	92.09	87.55	69.78	52.57	45.94	31.93
9.800	420.00	394.48	387.90	350.00	325.88	247.88	69.62	65.39	64.30	58.02	54.02	41.09

TABLE 2.

A. i. concentricus: fitted parameters for allometric relationships between clearance rate (CR, $\mu\text{L h}^{-1}$) or ingestion rate (IR, cells h^{-1}) and shell size (H, mm) or body weight (AFDW, mg).

Relationship	Parameter	1 $\text{c.}\mu\text{L}^{-1}$	5 $\text{c.}\mu\text{L}^{-1}$	10 $\text{c.}\mu\text{L}^{-1}$	20 $\text{c.}\mu\text{L}^{-1}$	30 $\text{c.}\mu\text{L}^{-1}$	50 $\text{c.}\mu\text{L}^{-1}$	Mean
CR = $a \times H^b$	<i>a</i>	2.183	1.889	1.483	1.051	0.775	0.554	2.460
	<i>b</i>	2.312	2.429	2.451	2.479	2.542	2.546	
	<i>r</i> ²	0.986	0.950	0.996	0.976	0.945	0.928	
CR = $a \times \text{AFDW}^b$	<i>a</i>	89.824	93.814	76.302	56.565	46.157	33.208	0.923
	<i>b</i>	0.868	0.912	0.920	0.931	0.954	0.956	
IR = $a \times H^b$	<i>a</i>	2183	9445	14830	21020	23250	27700	2.460
	<i>b</i>	2.312	2.429	2.451	2.479	2.542	2.546	
IR = $a \times \text{AFDW}^b$	<i>a</i>	89824	469068	763020	1131302	1384708	1660382	0.923
	<i>b</i>	0.868	0.912	0.920	0.931	0.954	0.956	

resources to meet their high metabolic demand during periods of low food supply. Larvae cannot afford to rely on their limited energy storage for as long as larger individuals do under unfavorable conditions.

The clearance rates of juvenile bay scallops determined in this study are comparable to the 42–96 mL mg^{-1} (dry weight [DW] per hour found for juveniles of *A. i. irradians* (calculated from Cahalan et al. 1989, assuming 30% is wet tissue, 80% of wet tissue is water) at comparable *I. galbana* concentrations and temperature. These values are much higher than that determined for juvenile *A. i. irradians*: 1.38–10.3 mL mg^{-1} (DW) per hour by Kuenstner (1988) (feeding on *Thalassiosira weissflogii*), and for adult bay scallops: 0.31–11.90 mL mg^{-1} (DW) per hour by Palmer (1980) and 1.3–8.9 mL mg^{-1} (DW) per hour (calculated, assuming 80% water content of tissue) by Chipman and Hopkins (1954). The higher weight-specific clearance rates determined for young juveniles in this study are in good agreement with the higher growth rate and metabolic rate (Lu 1996) measured for these early stages.

In the clearance rate–AFDW allometric relationships, the *b*-value is 0.923 ± 0.029 for bay scallop larvae and juveniles, which is much higher than the 0.584 (Kirby-Smith 1970) and the 0.82 (Chipman and Hopkins 1954, calculated by Winter 1978) determined for adult bay scallops, indicating that the clearance rate of juveniles increases much faster with increasing body size than that

of larger individuals. A similar trend was found in *M. edulis*, where the *b*-value was 1.03 for juveniles (Riisgård et al. 1980), but 0.66 (Möhlenberg and Riisgård 1979) and 0.72 (Riisgård and Möhlenberg 1979) for larger mussels. Mussel larvae often demonstrate *b*-values close to 0.8 (Jespersen and Olsen 1982, Sprung 1984). In oyster larvae, *b*-values were also found close to 1, e.g., 0.97 in *Crassostrea gigas* (Gerdes 1983) and 1.02 (Beiras et al. 1990) and 0.98 (Beiras and Camacho 1994) in *O. edulis*.

Clearance rate as a function of cell concentration determined in this study agrees well with those reported in other studies: it is high at low cell concentrations and decreases with increasing cell concentrations. In some experimental runs, clearance rates of juvenile bay scallops showed a reduction at 1 $\text{cell } \mu\text{L}^{-1}$ of *I. galbana*. Such a reduction at very low particle concentrations was also present for larvae of *M. edulis* (Sprung 1984), *M. mercenaria* (Riisgård 1988), and *O. edulis* (Beiras and Camacho 1994). A decrease of clearance rate at very low particle concentrations may serve to reduce energy consumption for the filtration process (Lam and Frost 1976, Lehman 1976). However, Sprung (1984) argued that the cilia of larvae have to move for larvae to swim, and thus, reduction in feeding cannot make a significant saving of energy. He postulated that the reduction in filtration activity was probably caused by errors resulting from processes such as contamination by dust, air bubble formation, and feces and mucus production of experimental animals.

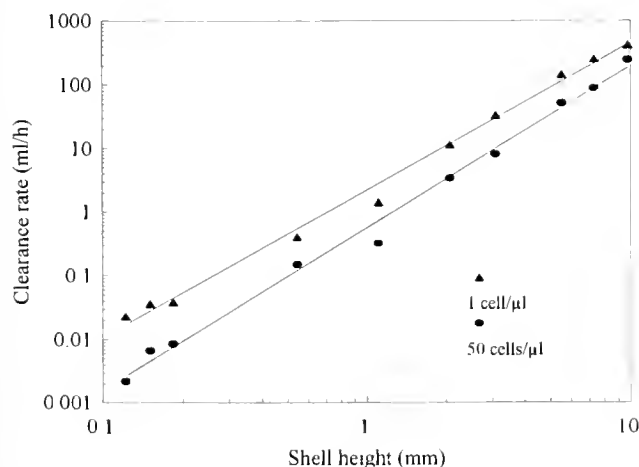


Figure 2. *A. i. concentricus*. Clearance rate of larvae and juveniles at two cell concentrations of *I. galbana* in relation to shell height.

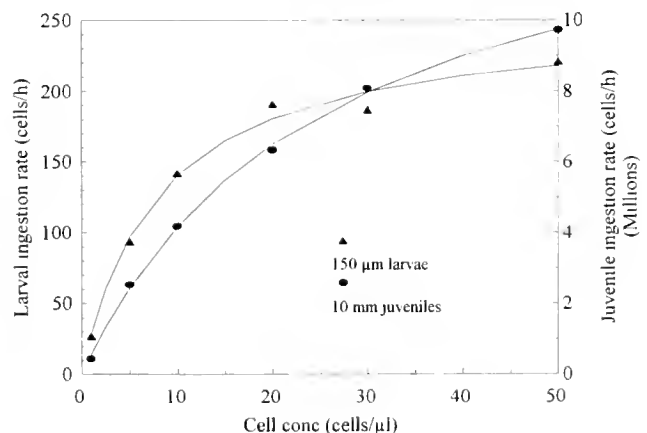


Figure 3. *A. i. concentricus*. Ingestion rate of 150- μm larvae and 10-mm juveniles in relation to *I. galbana* concentration (conc).

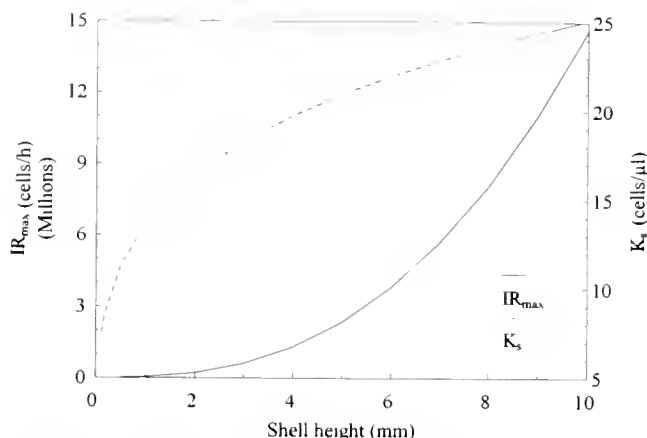


Figure 4. *A. i. concentricus*. Maximum ingestion rate (IR_{max}) and half-saturation concentrations (K_s) in relation to shell height.

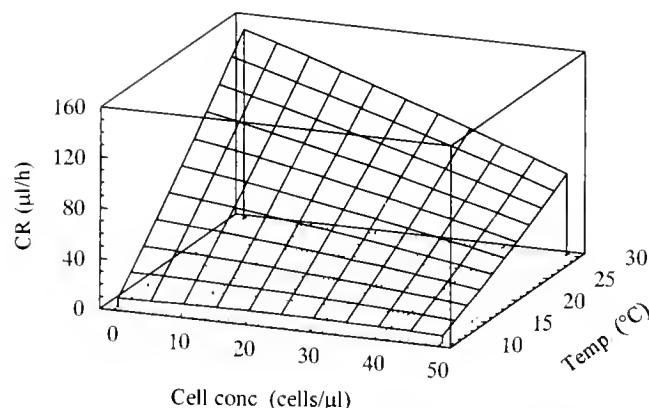


Figure 5. *A. i. concentricus*. Response surface plot of clearance rate (CR) of 5-mm juveniles to temperature (Temp., T) and cell concentration (conc., C) $CR = -58.6040 + 0.9810 \times C + 6.3150 \times T - 0.0030 \times C^2 + 0.0218 \times T^2 - 0.0853 \times C \times T$, $r^2 = 0.623$.

Ingestion rate shows a completely different pattern from that of clearance rate: a rapid increase in ingestion rate occurs at low cell concentrations, whereas the increase slows as cell concentration increases. Ingestion rate at high cell concentrations becomes relatively constant, indicating a maximum rate (the ingestion capacity) has been approached. The maximum rate is probably limited by the passage of food through the gut (Sprung 1984, Crisp et al. 1985). Winter (1978) stated that as the maximum ingestion rate was reached, the filtration rate decreased continuously in such a way that the amount of food ingested was kept constant and this pattern remained unchanged up to the food concentration at which animals began to produce pseudofeces. Such a plateau in ingestion was also observed for bay scallop larvae and juveniles in this study. The saturation points are at *I. galbana* concentration of 20 cells μL^{-1} for larvae and at >50 cells μL^{-1} for juveniles. The latter is in accordance with the >57 cells μL^{-1} determined for larger bay scallops (39.8–49.3 mm shell height) (Palmer 1980). It seems that larvae and small juveniles reach maximum ingestion rates at a lower cell concentration than juveniles of larger sizes. Such a trend can also be reflected in the values of K_s determined for the fitted ingestion rate–cell concentration kinetic curve, where K_s is an increasing function of juvenile size. The fact that larger bay scallops have higher saturation points than larvae and juveniles demonstrates that larger individuals are more capable of handling dense particle concentrations than are the early developmental stages.

In addition to the ingestion by the experimental animals, the production of pseudofeces may contribute to the loss of algal cells from the experimental media. This issue is rarely addressed in the literature on bivalve larvae, probably because the production of

pseudofeces in larvae is difficult to detect. It is also possible that the larvae may reject particles using their cilia (Strathmann et al. 1972) rather than producing pseudofeces. The relative importance of both mechanisms remains to be determined. The production of pseudofeces was not quantified and was variable between replicates in this study. The findings that pseudofeces were only observed in some of the tests, and more importantly, that pseudofeces were observed in one test and not in a follow-up test using the same animals under the same experimental conditions, demonstrate that the production of pseudofeces is not only just a function of the ambient cell concentration, but is also a process that may be related to the physiological condition of both the experimental animals and the algal cells. The production of pseudofeces by larvae and juveniles over the range of *I. galbana* concentrations (1–50 cells μL^{-1}) adopted in this study appears to be an exception. Cell reduction measured in the experimental media over the range of 1–50 cells mL^{-1} of *I. galbana* can be considered predominately as the result of ingestion, although it has been reported that the production of pseudofeces at much higher cell concentrations of another algae (0.55×10^6 to 1.46×10^6 cells mL^{-1} of chrysophyte *Aureococcus anophagefferens*) reached 25–35% of the algal cells filtered in bay scallops (Kuenstner 1988).

TABLE 3.

A. i. concentricus: clearance rates of 5-mm juveniles at different temperatures and cell concentrations (c.p.L $^{-1}$).

Temperature (°C)	Clearance rate (mL ind $^{-1}$ per h)					
	1 c.p.L $^{-1}$	5 c.p.L $^{-1}$	10 c.p.L $^{-1}$	20 c.p.L $^{-1}$	30 c.p.L $^{-1}$	50 c.p.L $^{-1}$
30	131.0	142.3	135.0	94.1	90.6	63.5
25	120.0	120.0	112.3	89.8	77.2	51.9
20	76.3	66.6	61.1	52.5	53.1	33.1
15	40.7	25.0	23.3	20.5	16.7	16.5
10	6.9	12.2	12.4	8.1	9.4	5.5

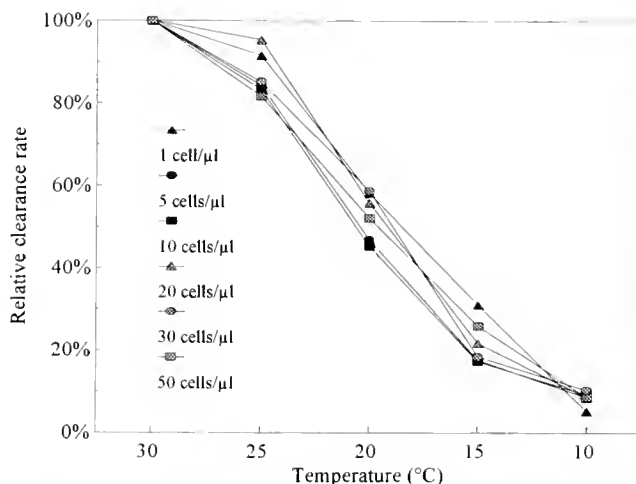


Figure 6. *A. i. concentricus*. Relative clearance rate of 5-mm juveniles vs. temperature at various *I. galbana* concentrations.

TABLE 4.

A. i. concentricus: analysis of variance for clearance rate and ingestion rate of larvae and juveniles.

Parameter	Source of Variation	Sum of Squares	d.f.	Mean Square	F-Ratio	Significance Level
Clearance rate (mL h ⁻¹)	Main effects					
	Temperature	45535.40	4	11383.90	62.14	0.0000
	Cell concentration	6514.22	5	1302.84	7.11	0.0006
	Residual	3663.75	20	183.19		
	Total (corrected)	55713.5	29			
Ingestion rate (million cells h ⁻¹)	Main Effects					
	Temperature	10.27	4	2.56	12.55	0.0000
	Cell concentration	10.11	5	2.02	9.88	0.0001
	Residual	4.09	20	0.20		
	Total (corrected)	24.47	29			

TABLE 5.

A. i. concentricus: multiple range analysis (95% LSD) for clearance rate and ingestion rate by temperature (homogeneous groups are marked by vertically aligned Xs).

Temperature (°C)	Count	Clearance Rate vs. Temperature		Ingestion Rate vs. Temperature	
		Least Squares Mean	Homogeneous Groups	Least Squares Mean (×10 ⁶)	Homogeneous Groups
10	6	9.07	X	0.15	X
15	6	23.78	X	0.36	X X
20	6	57.12	X	0.88	X X
25	6	75.19	X	1.42	X X
30	6	109.42	X	1.66	X

It has been well documented that clearance rate and ingestion rate are greatly influenced by temperature (e.g., Theede 1963; Schulte 1975; Kirby-Smith 1970; Bayne et al. 1976). In *M. edulis*, a decrease in temperature resulted in decreased clearance rates between 5 and 20°C (Bayne et al. 1976). Above 20°C, however, clearance rates declined. Earlier studies by Theede (1963) and Schulte (1975) also found the same trend for two separate mussel populations. Our results show that the clearance rate of juvenile

bay scallops is an increasing function of temperature between 10 and 30°C. The highest clearance rates were found at 30°C, which is close to the upper thermal tolerance limit of this species (Sastry 1961). This result differs from that of a study on larger bay scallops by Kirby-Smith (1970), who observed a relatively constant clearance rate for adult *A. i. concentricus* from 10 to 25°C. Such a different response in clearance rate to temperature between young and old bay scallops could indicate that larval and young juvenile bay scallops are more energetically adaptive to higher temperatures than adult individuals.

Clearance rate and ingestion rate of juvenile bay scallops increased slowly from 10 to 15°C, but rapidly at >15°C. Therefore, 15°C can be regarded as a critical temperature at which bay scallops change from a less active state to a more active state with respect to feeding activities. This is in accordance with the fact that in the natural habitat of bay scallops in central Florida, water temperature seldom drops below 15°C. For example, temperature ranges from 14 to 32.5°C in the Bayboro Harbor of Tampa Bay (Lu 1996) and from 12 to 30°C at Anclote Key, FL (Barber and Blake 1983). On the other hand, a rapid increase in clearance rate at >15°C probably reflects the adaptive strategy of the Florida bay scallop to the higher temperatures it generally experiences.

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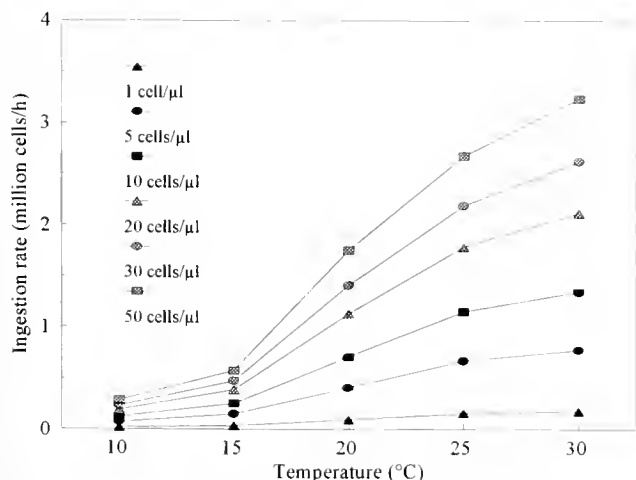


Figure 7. *A. i. concentricus*. Ingestion rate of 5-mm juveniles vs. temperature at various *I. galbana* concentrations.

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GENETIC DIVERGENCE AND LOSS OF DIVERSITY IN TWO CULTURED POPULATIONS OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS* (LAMARCK, 1819)

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ABSTRACT Researchers at the Virginia Institute of Marine Science (VIMS) have been maintaining a small-scale bay scallop (*Argopecten irradians*) culturing operation since the late 1960s. The cultured line was originally established with broodstock collected from the coasts of Virginia and North Carolina, but it has since been augmented with a "grab bag" of introductions from other source populations. A large bay scallop–culturing operation was reportedly founded in China in the early 1980s, with 26 individuals provided by the VIMS researchers. The degree of genetic divergence between these two populations since the founding of the Chinese operation is unknown, as are the relative amounts of genetic diversity that may have been maintained under the selective pressures of the hatchery.

Samples of cultured bay scallops were obtained from culturing operations in Wachapreague, VA, in 1993 and 1995, and from the Shandong Province of China in 1993. Mitochondrial DNA (mtDNA) was isolated from individual scallops, digested with a battery of eight restriction enzymes, and analyzed by restriction fragment length polymorphism analysis. Measures of haplotype diversity and divergence were calculated for the samples to reveal genetic differences between the cultured populations and to allow comparison of the levels of genetic variation maintained in the cultured populations relative to those observed in several natural populations of bay scallops. A sample of 55 Virginia cultured bay scallops was found to be monotypic, represented by a single haplotype, and three haplotypes were observed in 36 individuals sampled from China. No haplotypes were shared between the samples, indicating that significant divergence has occurred between the populations. The single haplotype from Virginia was observed in a sample of bay scallops from New England, and the least common haplotype from the Chinese sample was also found in samples from New England, North Carolina, and Crystal River, FL. Haplotype diversity and genotypic divergence values for the cultured samples indicate that mtDNA variation may be lost in the culturing process and that a bottleneck effect and/or genetic drift has affected the levels of variation in these populations differently. Assuming that the Chinese culturing operation was founded exclusively with individuals from the Virginia population, it can be concluded that the latter has lost a greater proportion of the original variation in the intervening generations of hatchery breeding.

KEY WORDS: Bay scallop, aquaculture, genetics, mtDNA variation, inbreeding

INTRODUCTION

The bay scallop, *Argopecten irradians* (Lamarck), is endemic to shallow estuarine habitats along the East Coast of the United States, from Massachusetts to Texas (Clarke 1965). Four subspecies have been described based on shell morphometrics (Waller 1969, Petuch 1987), although both restriction fragment length polymorphism (RFLP) analysis of mitochondrial (mt) DNA (Blake and Graves 1995) and allozyme studies (Marelli et al. 1997) have indicated that individuals described as the subspecies *A. i. taylorae* are genetically indistinguishable from *A. i. concentricus*. The bay scallop has been fished commercially since the mid-1800s, and it also supports a large recreational fishery (Shumway and Castagna 1994). Because populations appear to be recruitment limited (Peterson and Summerson 1992) and highly variable in size, the potential for aquaculture of the species has received considerable attention.

The first significant attempt to rear cultured bay scallops to market size was undertaken by Castagna and Duggan (1971) in the late 1960s. The initial stock for the study consisted of 66 adult bay

scallops (*A. i. concentricus*) collected from bays along the Eastern Shore of Virginia and from Bogue Sound, NC. In the years since the establishment of this original cultured line, the broodstock has been supplemented with individuals from Massachusetts (*A. i. irradians*) and Texas (*A. i. amplicostatus*) (M. Castagna, Virginia Institute of Marine Science [VIMS] 1993, pers. comm.). These additions served to contaminate the line so that the exact subspecific composition of the current broodstock is unknown. This bay scallop–culturing effort has continued to the present day, coordinated by researchers from VIMS. A typical spawning protocol involves a mass, induced spawning of 100–200 broodstock animals, to produce an estimated 50–150 million eggs. Several such spawns may be performed and the resultant eggs pooled. A small commercial market has developed for the Virginia cultured product.

In 1982, 128 scallops from the VIMS Eastern Shore culturing operation were transported to laboratories in Qingdao, China, with the intent of establishing a bay scallop–culturing effort in the waters of China's eastern bays. Twenty-six of the transported individuals survived the journey to spawn in January 1983 (Chew 1990). By 1989, Chinese production of the "Virginia" American bay scallop exceeded 50,000 metric tons in-shell live weight (Chew 1990).

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The potential for loss of genetic variability due to inbreeding seems great for both the Virginia cultured line and that maintained in China. The relative degree and possible consequences of this loss are unknown. *A. irradians* is a functional hermaphrodite, and many of the larvae produced in the hatchery may be the result of facultative selfing. Inbreeding depression has been observed in self-fertilized larvae of the catarina scallop, *Argopecten circularis*, manifest as decreased larval growth and lower rates of survival (Ibarra 1995). Such inbreeding effects are thought to be a general danger for cultured species with very high fecundities, in which few individuals may produce large numbers of offspring (Newkirk 1978). The effective population size (N_e), or the number of broodstock individuals contributing gametes to the subsequent generation, may in fact be much smaller than the census number (N) of individuals used as broodstock in a hatchery (Gaffney et al. 1992). Culturing techniques in which parental individuals are mass spawned may exacerbate inbreeding problems, even when the number of resultant progeny is satisfactory.

By 1993, the Virginia and Chinese broodstocks had been isolated for 10 y and at least 10 generations, a period that should have permitted effects of the founding event and genetic drift to become apparent. It has been shown that RFLP analysis of the mtDNA reveals considerable genetic variation in natural populations of the bay scallop and that geographically isolated populations are genetically distinct (Blake and Graves 1995). In this study, a comparison of mtDNA variation in the Chinese and Virginia cultured populations, and in samples from bay scallop populations in their natural range, was undertaken to determine the genetic divergence between the Chinese and Virginia populations and the level of genetic variation maintained in the cultured populations relative to that in natural populations.

MATERIALS AND METHODS

A sample of 27 cultured bay scallops was provided by the VIMS laboratory at Wachapreague in March 1993. These were year-old individuals, progeny of broodstock spawned in April 1992. An additional sample of 28 individuals was obtained from the facility in March 1995. These were products of the April 1994 spawn and permitted comparison of temporally isolated samples of the Virginia cultured scallops.

Fresh tissue from 36 cultured bay scallops—18 from a northern growout site (Laizhou) and 18 from a growout site in Qingdao (Tiaonan)—was obtained from Chinese culturing facilities in October 1993. Although these individuals had been reared at the different sites, the seed originated from the same broodstock (X. Qinzha, Institute of Oceanology, Academia Sinica, 1993, pers. comm.). Dissection of tissues from Chinese bay scallops was performed by the investigator (SGB), and confirmation that all were *A. irradians* was made at this time. The indigenous Chinese scallop, *Chlamys farreri*, is easily distinguished from the bay scallop, and none was included among the sampled individuals.

MtDNA was purified from scallop gonad, mantle, and gill tissue by cesium chloride density-gradient ultracentrifugation, as described in Blake and Graves (1995). The difficulties of transporting usable tissue from China to the United States made it necessary for initial preparative steps to be taken in the laboratories of the Institute of Oceanology in Qingdao, China. DNA isolation was initiated in China in early October 1993, but because equipment for ultracentrifugation was not available at this facility, the samples were maintained on ice (or orange-flavored ice pops,

when ice was unavailable) after the addition of CsCl-saturated water to the tissue preparations (see Blake and Graves 1995). The samples were then transported to VIMS, where mtDNA was purified by cesium chloride density-gradient ultracentrifugation.

Purified mtDNA was digested with a battery of eight restriction enzymes for all individuals: *Ava*I, *Ban*I, *Ban*II, *Bgl*II, *Bst*EII, *Eco*RI, *Hae*II, and *Hind*III. Restriction fragments were end-labeled with the Klenow fragment of DNA polymerase I and 35 S-labeled nucleotides, electrophoresed at 1 V/cm in 1% agarose gels overnight, and visualized by autoradiography (Sambrook et al. 1989). 35 S-labeled 1-kilobase ladder DNA (BRL) provided a molecular-weight size standard.

Sizes of mtDNA fragments were estimated by fitting band migration distances to those of the standard by the local reciprocal method of Elder and Southern (1983) by use of the program Gel Frag Sizer (Gilbert 1989). Restriction sites were inferred from completely additive fragment patterns, and letter designations were assigned to the different patterns. Eight-letter composite haplotypes were compiled for the series of enzymes and analyzed for site changes, following Blake and Graves (1995).

Statistical analyses were performed with the Restriction Enzyme Analysis Package (REAP) (McElroy et al. 1991). For each sample, haplotype and nucleotide diversities were calculated following the methods of Nei (1987) and Nei and Miller (1990), respectively. Mean nucleotide sequence divergence between samples was calculated following Nei and Miller (1990) and was corrected for within-population polymorphism by subtracting the average of within-sample diversities. Because several of the haplotypes observed were rare, a Monte Carlo simulation (Roff and Bentzen 1989) was performed to estimate heterogeneity and assess the likelihood that the sampled populations shared a common gene pool. Data from natural bay scallop populations (Blake and Graves 1995) were also used in comparative analyses with the Chinese and Virginia cultured bay scallop samples, to assess changes in diversity and divergence under culturing conditions.

RESULTS

DNA from a total of 91 cultured bay scallops was analyzed with eight restriction endonucleases, revealing four distinct mtDNA haplotypes (Table 1). The 1993 and 1995 samples of cultured bay scallops from Virginia were both monotypic, characterized by a single haplotype, AABAAAAE, and the two were combined into a single pooled sample (VA) of 55 monotypic individuals for further analysis. The haplotype diversity and mean nucleotide sequence diversity for the Virginia population were both calculated to be zero. The combined cultured Chinese sample (Q) comprised three haplotypes, none of which was identical to that observed in the Virginia sample.

A Monte Carlo test for heterogeneity was performed (Roff and Bentzen 1989) on the two subpopulations of bay scallops from China (Laizhou and Tiaonan), to determine whether these shared a common gene pool (originated from a common broodstock) and could be treated in subsequent analyses as one population. One thousand Monte Carlo randomizations yielded 126 χ^2 values exceeding the value from the original data, indicating that at $p = 0.126$, the populations are not significantly heterogeneous. The Chinese sample is hereafter discussed as a single population.

For the Chinese sample, haplotype diversity was 0.55 and mean nucleotide sequence diversity was 0.33%. The two less common haplotypes in the Chinese sample, ACCAAAAA and

TABLE 1.

A. irradians: composite haplotypes from two populations of cultured bay scallops and numbers of these haplotypes observed in five samples representing natural bay scallop populations.

Haplotype	Δ	Cultured		"Natural"			
		Q	VA	MA	NC	FL	RK
AABAAAAE	0		55	5			
AACAAAAA	2	4 (4)		7	26	2	
AABAAAE	1	22 (9)					
ACCAAAAA	3	10 (5)					
Total n		36	55	26	48	27	34

Q, Qingdao, China; VA, Wachapreague, VA; MA, New England; NC, Harker's Island, NC; FL, Crystal River, FL; RK, Rabbit Key, FL. Restriction enzymes used: *Ava*I, *Ban*I, *Ban*II, *Bgl*II, *Bst*II, *Eco*RI, *Hae*II, and *Hind*III. Values in parentheses are totals from the Laizhou, China, growout facility. Δ is the number of site changes between the haplotype and the arbitrary standard of the single Virginia haplotype. A complete list of the haplotypes from the "natural" samples is provided in Blake and Graves (1995).

AACAAAAA, differed from each other by a single site change, whereas the third and most common, AABAAAE, differed from these by several site changes. The latter, however, differed from the Virginia haplotype (AABAAAAE) by only one site change. The corrected mean nucleotide sequence divergence between the Virginia and Chinese samples was 0.13%.

Because there were no shared haplotypes between the Virginia and Chinese samples, it was not necessary to apply a rigorous test for heterogeneity to these two populations. Data from other bay scallop populations (Blake and Graves 1995) were used for comparison with the cultured samples of this study. Included in analyses were samples of natural populations from Harker's Island, NC, and Rabbit Key, FL. Hatchery-reared scallops from Woods Hole, MA (New England), and Crystal River, FL (Florida Gulf), were used to approximate genotype distributions for their regions of origin. These were not used in comparisons of genetic diversity. An abbreviated list of the genotypes found in the "natural" populations, including those found also in one of the cultured samples, is presented in Table 1.

Tests for heterogeneity were performed between the cultured samples and the sample from New England (Blake and Graves 1995), with which each shared a single haplotype. In both tests (MA and VA, and MA and Q), the 1,000 randomizations produced no χ^2 values higher than the observed, indicating that significant heterogeneity exists between the tested pairs. The least common haplotype from the Chinese sample (AACAAAA), represented by four individuals, was also present in the New England, North Carolina, and Crystal River samples. No other haplotypes were shared between the cultured samples and those representing natural bay scallop populations. Two of the three Chinese haplotypes were unique to that sample.

Corrected mean nucleotide sequence divergences between the cultured samples evaluated in this study and the natural populations previously described (Blake and Graves 1995) ranged between 0.04% (Q vs. MA) and 0.24% (Q vs. FL) (Table 2). Although still not sharing a common gene pool, the Chinese sample was found to be less divergent from the New England sample (0.04%), with which it shared a single common haplotype, than from the cultured Virginia population (0.13%).

DISCUSSION

The genetic aspects of hatchery rearing of bay scallops are of great interest to culturists in the United States, where the bay scallop is native, and China, where culture of this scallop is being undertaken on a very large scale. A loss of genetic variation due to drift is apparent in both the Chinese and the Virginia cultured lines, although most notably so in the latter, which has apparently become fixed for a single mtDNA haplotype. The Chinese population represented by the Qingdao sample also possessed a lower haplotype diversity (0.55) than natural bay scallop populations from Rabbit Key (0.91) and North Carolina (0.69, pooled) (Blake and Graves 1995). The strategy of broadcast spawning appears to be very effective at maintaining genetic diversity for the bay scallop in nature.

It can be stated with confidence that at the founding of the Virginia line, there was a greater level of genetic diversity present in the broodstock than was measured in this study. MtDNA analyses of a natural population from North Carolina, one of the putative sources of the Virginia line, revealed considerably higher haplotype diversities (Blake and Graves 1995). In two samples from North Carolina, the haplotype diversity, or probability of encountering different haplotypes when two individuals are sampled from a population, ranged between 0.63 and 0.74. Even if hatchery rearing has reduced this level in the Virginia cultured line, periodic introductions from other source populations should have served to reintroduce genetic variability to the population.

The samples obtained from the Virginia culturing facility originated from mass spawnings of 100–200 animals. The numbers of contributing parent individuals are not precisely known, but it is apparent from the current monotypic state of the population that differential reproductive success has occurred during one or more of these spawning events. A single cataclysmic loss may have occurred in which one or very few maternal individuals contributed to the subsequent generation. Similarly, a series of less dramatic losses may have occurred, to bring the population to fixation over a period of generations.

The initial bottleneck of no more than 26 breeding individuals that established the Chinese culturing operation in 1983 was not sustained, because the production of bay scallops in China grew extremely rapidly. If the 26 transplanted individuals reflected all of the genetic variation present in the Virginia source population at the time, it would appear that Chinese culturing methods have been more conducive to a maintenance of that variation. This tendency for loss under the Virginia hatchery regimen may be even more pronounced, if subsequent additions have been made to the Vir-

TABLE 2.

A. irradians: matrix of nucleotide sequence divergences among populations, in percents, corrected for within-sample variation.

	VA	Q	MA	NC	FL
Q	0.13				
MA	0.06	0.04			
NC	0.32	0.18	0.12		
FL	0.31	0.24	0.18	0.14	
RK	0.21	0.15	0.12	0.19	0.11

VA, Wachapreague, VA; Q, Qingdao, China; MA, New England; NC, Harker's Island, NC; FL, Crystal River, FL; RK, Rabbit Key, FL. Values in boldface represent samples from this study.

ginia broodstock since the founding of the Chinese line. The number of individuals spawned to produce the sampled population from China is not known but, based on the relative magnitude of the operation, is presumed to be higher than that used in Virginia. It may be that it is simply the scale of the operation that leads to a greater maintenance of diversity. That is, the Chinese may be maintaining multiple lines with low or no diversity, rather than one. Conversely, it may be that instead of fewer mass spawnings, progeny from many, relatively small spawning events are pooled, as recommended by Gaffney et al. (1992), to prevent loss of variation by genetic drift.

Genetic divergence between the two cultured populations is difficult to assess, particularly given the monotypic character of the Virginia samples. Cultured bay scallops from China were not found to share a common gene pool with the putative Virginia source population, and given the lack of any shared mtDNA haplotypes, this finding is not surprising. Corrected mean nucleotide sequence divergences (Table 2) indicate that the bay scallops in the Chinese sample were least divergent from those in the New England sample. It is likely that at the time the scallops were sent to China, the Virginia population also contained a genetic component resembling that found in New England. The introduction of New England bay scallops into the Virginia broodstock is known to have taken place, although it has generally been assumed that the majority of the stock originated from animals that set naturally off North Carolina and Virginia's Eastern Shore. The presence in the Chinese sample of two haplotypes not found in the other sampled populations may also indicate that some rare genotypes, missed in the sampling of the natural populations, were present in the broodstock sent to China but disappeared in the Virginia line. The lack of a sample from the putative Texas source population (from which introductions were made to the Virginia line) makes the

possible presence of Texas genotypes in the Chinese sample impossible to evaluate.

It is difficult, in conclusion, to say much about mtDNA variation in either of these cultured lines beyond what can be measured in the current population, because there are periods in the development of both in which the origin of broodstock or the methods of breeding are unclear. This lack of information underscores the importance of good hatchery recordkeeping, and the loss of diversity in both cases highlights the need for a careful breeding regimen that maximizes the number of parental contributors in a broodstock. If one or few spawnings are used to replace the Virginia broodstock for the subsequent generations, then a monotypic lineage will likely persist and problems associated with inbreeding depression may become more apparent. This may also be a problem, more slow to develop but likely more catastrophic to the industry if it does occur, in the Chinese culturing operation.

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SOUTHWESTERN ATLANTIC SCALLOP (*ZYGOCHELAMYS PATAGONICA*) FISHERY: ASSESSMENT OF GEAR EFFICIENCY THROUGH A DEPLETION EXPERIMENT

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ABSTRACT Fishing gear (demersal otter trawl) efficiency in a scallop fishery (*Zygochlamys patagonica*) of the southwestern Atlantic was estimated through a depletion experiment. During the autumn of 1995, a plot (1.257 km²) was delimited (using satellite-derived coordinates) within a scallop bed (42°15'S, 58°33'W), and a 56-m scallop boat performed 89 tows (in a 3-day period), sweeping an estimated area of 2,796 km² (2.2 times the size of the experimental plot). During the experiment, the catch per unit of effort dropped to 52% of its initial value. Catchability (q_b) and initial biomass in the experimental bed were estimated by the use of depletion methods. Estimates of mean biomass density ranged between 0.23 (Leslie method) and 0.22 kg of scallops · m⁻² (DeLury method). On the basis of the depletion experiment, and defining $F = q_b \cdot f = (a/A) \cdot e \cdot f$ (a , area swept by a unit of fishing effort; A , total fishable area of the experimental plot; e , gear efficiency; f , effort) for the experimental bed, e could be calculated when the area swept by a unit of effort is known. The estimated efficiencies of the net using this relationship were in the range of 21–31%, which is in the upper range of values estimated for most scallop fisheries using dredges, but less than that recorded for scallop fisheries that use trawl gear.

KEY WORDS: Gear efficiency, scallop, *Zygochlamys patagonica*, fishery

INTRODUCTION

The scallop *Zygochlamys patagonica* is an abundant species distributed along the Magellanic province: on the Chilean coast from about 42°S to Tierra del Fuego, and on the Argentinean shelf north to the estuary of the La Plata River (35°S), at depths ranging between 60 and 175 m (Waloszek 1991, Lasta and Zampatti 1981). Maximum shell height is 79 mm, and sexes are separated (Waloszek and Waloszek 1986; but see Orensanz et al. 1991 for contradictory evidence). Sexual maturity is reached at 45 mm shell height (≈2 y old), and spawning takes place during spring and late summer to early fall (Orensanz et al. 1991).

Previous evidence suggests that stocks of this species may be very large (Waloszek 1991, Orensanz et al. 1991), although because of the small size of the adductor muscle, hand processing onboard was thought to be impractical (Orensanz et al. 1991). Furthermore, potential fishing grounds are located in offshore waters, which are generally beyond the working range of the small inshore fleet that operate on the tehuelche scallop (*Aequipecten tehuelcha*; Orensanz et al. 1991). The incorporation of onboard automatic processing techniques to scallop fisheries (i.e., Iceland scallop, *Chlamys islandica*) and the incorporation of larger boats in the Argentinean fleet have generated renewed interest in developing a fishery for this species. One fishing vessel landed 1,313 metric tons of meat (pers. obs.) during an experimental fishing season in 1995. However, major gaps in the knowledge of this species exist. Among these omissions are estimates of absolute abundance.

To estimate abundance based on sweep area methods, it is important to evaluate gear efficiency (e , the fraction of the animals present in the path of the gear that are actually captured). The efficiency of scallop fishing gears has been assessed by several means (quadrat samples of dredged and undredged areas: Caddy 1968, Shafee 1979; underwater TV surveys: Mason et al. 1982,

Giguère and Brulotte 1994; tagging experiments: Dickie 1955, Gruffydd 1972). Alternatively, gear efficiency estimation based on depletion methods can be made more general by encompassing larger areas, therefore avoiding variability in the spatial distribution of scallops within the bed (Joll and Penn 1990). These methods have been successfully used in the tehuelche scallop *Aequipecten (Chlamys) tehuelcha* dredge fishery (Iribarne et al. 1991).

The primary objective of this work is to estimate gear efficiency of the bottom trawl net used in this developing scallop fishery. The study is based on a depletion experiment conducted in the southwestern Atlantic (42°15'S, 58°33'W; 180 miles off the Argentinean coast).

MATERIALS AND METHODS

The fishing gear used in this experiment (also used in the developing fishery) was a bottom otter trawl similar to the gear used in the calico scallop fishery (*Argopecten gibbus*). This gear had a total length of 13 m. The otter boards were conventional rectangular, steel-framed doors with timber panels, 1 m in height, 3.4 m long, and weighing 490 kg each. Doors were attached to a single tow wire (or trawl warp) by a 26-m-long bridle. The head rope and the foot rope (made of 1.9-cm-diameter rope) were 15 m long and directly attached to the doors (otter boards). There are two tickler chains (4.3 kg · m⁻¹ each) attached to the foot rope. The net was constructed of 6-mm polypropylene twine with 10-cm mesh size. The cod-end was made of 8-mm nylon twine with a 10-mm mesh size. The top and bottom panels were identical, but the bottom had attached pulley chaffing gear to protect the net. The net's path width was estimated by Garcia and Ercoli (1996), following the procedures suggested by Tauti (1963). By this procedure, the net drag under an average velocity of 3.85 knots was $R_r = 1,040$ kg, the door's resistance was $r_x = 465$ kg, and the

spreading force was $\gamma = 530$ kg. Using an iterative procedure, García and Ercoli (1996) showed that the net mouth opening is 12.6 m ($Ah = 0.84 \lambda = 12.6$ m; Ah , net path width; λ , headline length) when the net was empty and 10.6 m with a load of 1,200 kg, which was the average load found in our study (half of the average load gives an estimation of 11.5 m). The scallop boat (56 m long, two 1,120-horsepower engines) normally operated with two nets, towed by a cable and using a length-to-depth ratio of 3:1. The null hypothesis of no difference in the capture per haul between the two nets was evaluated by use of a paired *t*-test (Zar 1984).

A 1.257-km²-area (parallelogram-shaped) experimental plot was located by satellite coordinates (precision of ± 40 m) by a procedure similar to that of Joll and Penn (1990). The area was selected because of high scallop density, after an initial exploration of the fishing ground. The bottom was homogeneous, composed of fine sand with a depth ranging between 90 and 105 m. The experiment lasted 3 days (March 5–7, 1995). During the fishing operation, we recorded the location of each trawl start and end point, towing time and speed, unsorted catch per tow, and direction of the tows. To randomize fishing hauls within the experimental plot, the starting point of each tow was chosen randomly before the commencement of the experiment.

The unsorted catch (scallop and bycatch) per tow was calculated by visually estimating the extent to which the cod-end was filled, based on categories of 10% before it was opened on deck. The relationship between catch weight and extent of cod-end fullness was investigated before field trials commenced, and a linear relationship between the extents of cod-end volume (or filling) was assumed. The catch weight for different proportions of cod-end fullness (20, 50, 80, or 100%) was evaluated before the experiment and provided the following relationship: $a = 22.98$ (SE = 1.37), $b = 4.28$ (SE = 98.89), $r^2 = 0.95$, $n = 17$. Then, on the basis of these data, the proportion of cod-end filling was transformed to weight on the basis of a linear relationship between cod-end percentage fullness and capture weight. A full net was estimated to contain 2,298 kg (SD = 294 kg, $n = 17$) of scallop + bycatch. Then, the amount of scallop catch (in kg) was estimated by randomly taking a 10-kg sample from 72 tows (80.1% of the tows during the experiment). This sample size was decided on the basis of the time and space available onboard for sampling. This sample was weighed (0.1 kg accuracy). Catch per unit of effort (CPUE) was expressed as scallop weight (in kg) captured by towing time (in hours) per net. Fishing distance was estimated on the basis of towing time and speed, calculated with the help of the General Positioning System equipment. The size of scallops captured during the experiment ranged from 28 to 80 mm shell height.

The initial abundance of scallops in the experimental plots and the catchability coefficients (q) were estimated by the use of removal methods (Ricker 1975). These methods assume a closed population and constant q ; thus, abundance on a given fishing ground declines only as a consequence of fishing. The data were fitted to both Leslie and DeLury models by the use of linear regression. The models are as follows:

$$\text{Leslie model: } C_t/f_t = q \cdot N_i - q (K_t + C_t/2)$$

$$\text{DeLury model: } \ln (C_t/f_t) = \ln (q \cdot N_i) - q (E_t + f_t/2)$$

where C_t/f_t is CPUE over time period t , N_i is initial population abundance, N_t is abundance at the beginning of time period t ,

K_t is cumulative catch taken before that time period, E_t is total fishing effort applied before time period t , and \ln is natural logarithm.

Given that abundance was expressed in terms of biomass, we assume either that growth and natural mortality was insignificant over the duration of the experiment, or that growth and natural mortality balanced each other out. This is a reasonable assumption, given the short experimental time (3 days). All evidence suggests that the other assumptions implicit in the use of these models (closed population: no emigration, immigration, mortality, or recruitment) were satisfied. The parameters of both models were estimated by means of linear regression. The assumptions of the two methods differ: CPUE is normally distributed in the Leslie model and log normally distributed in the DeLury model. Errors in the observation of the independent variable (Leslie, cumulative catches; DeLury, cumulative efforts) are assumed to be negligible.

According to Caddy (1979), fishing mortality (F) in this type of fishery can be expressed as $F = q \cdot f = (a/A) \cdot e \cdot f$ (a , area swept by a unit of fishing effort; A , total fishable area of the experimental plot; e , gear efficiency; f , effort). Given that we refer to fishing mortality and catchability within an experimental patch of scallops, rather than using the entire stock, we will name these values as F_b and q_b . Because a and A are known, e can be calculated using q_b estimated from the depletion experiment. The value a is assumed to be constant, and its estimation is based on a constant door-to-door distance during the fishing operation. The variance of e was estimated by assuming that a and q are random variables (Iribarne et al. 1991). Nonparametric bootstrap techniques (Efron 1982) were used to estimate median values, standard errors, and confidence intervals (CI) for the three estimated parameters (N_i , q_b , and e). In each bootstrap sample, the value of the dependent variable (y) was recomputed for each value of x , by adding to the predicted y value a residual obtained with replacement from the set of residuals produced by the original regression (see Iribarne et al. 1991 for similar application). Then, using linear regression, a combination of (N_i , q_b) estimates were then produced using these recomputed y values. An estimate of e was obtained from this set of (N_i , q_b) estimates using a value of a randomly selected from a normal distribution, with mean and variance fixed at their estimated values. Median and 95% confidence limits for the three estimates were obtained from the distribution of (N_i , q_b , e) that resulted from 1,000 bootstrap replications. This nonparametric procedure avoids the assumptions of normality of residuals made in conventional linear regression.

RESULTS AND DISCUSSION

The scallop catch from the experimental plot was 144.2 metric tons, obtained from 89 fishing operations (79% of them with two nets) in three fishing days. There was no significant difference in the catch of the two fishing nets (average difference = 139 kg, SE = 189 kg, $n = 76$) when operating simultaneously ($t_{\text{paired}} = 0.735$, $df = 75$; $t_{0.05(2)} = 1.993$, $P > 0.05$). Therefore, the catch rate from both nets was used to calculate CPUE. The proportion of cod-end filling averaged 51% (SE = 13%, $n = 89$). Total fishing effort was 32 h and 40 min of towing time (per net). Estimated mean fishing speed was $8.0377 \text{ km} \cdot \text{h}^{-1}$ (SE = $1.0927 \text{ km} \cdot \text{h}^{-1}$, $n = 89$). Assuming a net opening of 10.6 m, the estimated area swept by one unit of effort (one net during 1 h) was $a = 0.0856 \text{ km}^2 \cdot \text{h}^{-1}$ (SE = $0.0102 \text{ km}^2 \cdot \text{h}^{-1}$). Total area swept over the whole experiment was 2.796 km^2 (2.2 times the size of the ex-

perimental plot). However, if the estimation were performed with half of the average catch (600 kg), the net opening would be 11.5 m, and the estimated area swept by one unit of effort is $a = 0.0928 \text{ km}^2 \cdot \text{h}^{-1}$ (SE = $0.0113 \text{ km}^2 \cdot \text{h}^{-1}$). In this case, the total area swept over the whole experiment is 3.032 km^2 (2.4 times the size of the experimental plot). The assumption of a constant net opening is a matter of discussion, but several lines of evidences suggest that it is a reasonable assumption in our study. Although this is a value with high variability that is difficult to estimate in trawl bottom fishing gears (see Gunderson 1993), it has been shown that when bridles are strapped together in front of the doors, there is a remarkable increase in the constancy of the doorspread at any depth (see Engås and Ona 1991). Our gear should work in similar way, because bridles are short and merge into one tow wire. Thus, we believe that it is safe to assume a constant opening. Furthermore, because our experiment was restricted to an area where depth was constant, the variability produced by the warp/depth ratio on the door opening (see Koeller 1991) was avoided. In any case, we believe that it will be constructive to study the behavior of this type of nets, under different loads and depth regimens.

During the experiment, CPUE (yield of scallops per hour) fell to 52% of its initial value (Fig. 1). Estimates of catchability and initial biomass (IB) obtained by the Leslie model ($q_b = 0.01992$, SE = 0.00333; IB = 297 tons, 95% CI-254-365 tons) and the DeLury model ($q_b = 0.02003$, SE = 0.00350; IB = 285 tons, 95% CI-243-345 tons) were similar (t -test for $q_b = 0.22$, $df = 88$, $p > 0.05$). These figures correspond to 0.19–0.28 kg of scallops $\cdot \text{m}^{-2}$. Estimated efficiency, depending on the load assumed, was in the range of 23–34% (Leslie: $e = 29.2$, 95% CI = 23.8–33.8; DeLury: $e = 29.4$, 95% CI = 23.6–34.1) when average load was assumed and 21–31% (Leslie: $e = 26.9$, % CI = 21.9–31.1; DeLury: $e = 27.1$, 95% CI = 21.8–31.5) when we used one-half of the average load.

The catchability coefficients estimated in our study by two methods are not statistically different. Thus, the estimated values could be taken as robust estimates. These values of gear efficiency are rather high (e , 21–31%) when compared with that estimated for the scallop dredge commonly used in the southwestern Atlantic (*A. tehuelcha* 15–21%; Iribarne et al. 1991) and lower when compared with trawl efficiency (60–64%) estimated by Joll and Penn (1990) for the scallop *Amusium balloti*, but it is in the same range of values of many scallop dredges used worldwide. These values range from 2 to 78% (i.e., *Placopecten magellanicus*: 5–20% Digby bay dredge, Dickie 1955; 8–78%, Giguère and Brulotte 1994; 2.1% 8-foot dredge, Jamieson 1978; 0.6–8.3%, offshore dredge; *Pecten maximus*: 13.4–35%, Gruffydd 1972, Mason et al. 1982, Mason et al. 1979; *Pecten fumata*: 26–62%, Gwyther et al. 1986, Gwyther and McShane 1984, Butcher et al. 1981; *Chlamys varia*: 6.7–28.3%, Shafee 1979; and *Chlamys opercularis*: 17%, Dupouy and Latrouite 1976). However, the values are still low and

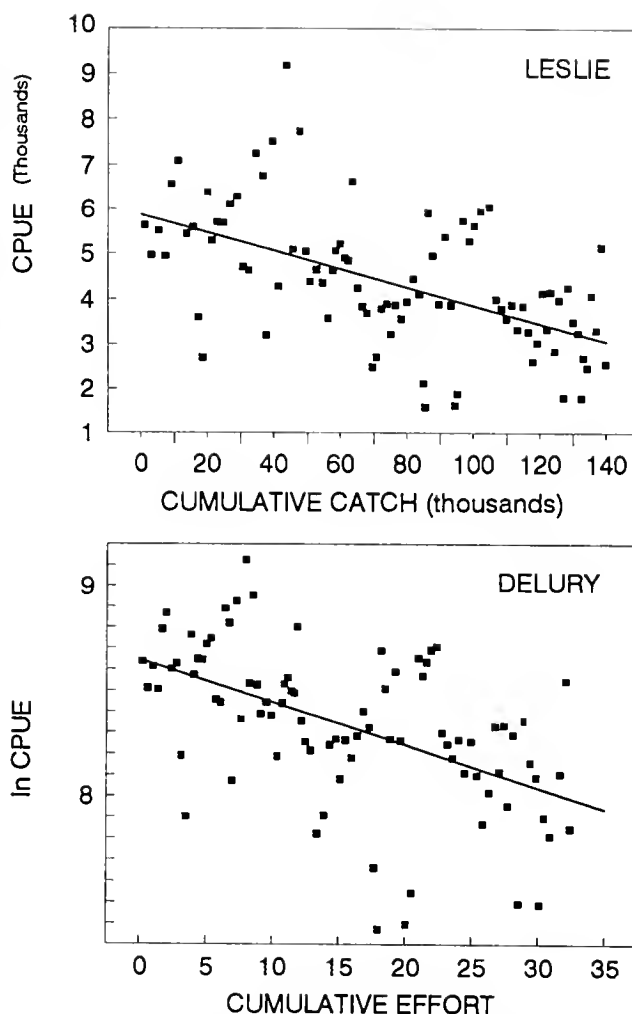


Figure 1. (a) Leslie method: (CPUE $\text{kg} \cdot \text{h}^{-1}$) against cumulative catch (in kg). (b) DeLury method: natural logarithm of CPUE ($\text{kg} \cdot \text{h}^{-1}$) against cumulative effort (towing time in hours). Each point represents one haul.

biomass estimation based on fishery surveys should take into account these low gear efficiencies.

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SURVIVAL OF SAUCER SCALLOPS, *AMUSIUM JAPONICUM BALLOTI*, AS A FUNCTION OF EXPOSURE TIME

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ABSTRACT The use of size limits for saucer scallops, *Amusium japonicum balloti*, presupposes that undersized scallops returned to the water survive the stress of capture and subsequent exposure to air while being sorted and graded. A tagging experiment, conducted to assess the survival of saucer scallops exposed to air for varying periods of time, showed that scallops exposed to air for periods of up to 120 min (2 h) were recaptured at the same rate as controls exposed to air for less than 2 min. Scallops exposed for periods longer than 150 min were recaptured at significantly reduced rates. These results suggest that saucer scallops can survive exposure to air for up to 2 h without suffering significant mortality and that the current use of size limits is justified in the context of maximizing value per recruit.

KEY WORDS: *Amusium*, scallop, survival, exposure

INTRODUCTION

Saucer scallops, *Amusium japonicum balloti*, support a trawl fishery off the Queensland (Australia) eastern coast from which annual landings average approximately 1,200 tons of adductor meat. The Queensland saucer scallop stock appears to be exploited heavily (Dredge 1988), although a stringent management regime appears to have reduced the risk of recruitment overfishing (Dredge 1992). The fishery is regulated through a range of input controls, as a means of both maintaining breeding stock levels and maximizing value per recruit.

Size limits are one of the range of management measures used in the fishery's management (Dredge 1992). Saucer scallops are normally landed whole, for shucking in shore-based factories or in designated near-shore shucking zones where size limits can be policed. They are subject to a size limit of 90 mm (shell height [SH]) in summer and early autumn (November to April inclusive) and 95 mm SH in late autumn, winter, and spring (May to October). The differences relate to variations in spawning and the adductor muscle condition of the scallop (Dredge 1981). By having a larger size limit in autumn and winter months, fishery managers planned to reduce exploitation during the winter spawning season, when adductor condition is at its poorest, and thus maximize value per recruit (Dredge 1994).

Participants in the fishing industry have expressed concerns about the effectiveness of these measures, particularly in relation to the process of holding scallops while they are accurately measured. Some fishermen have suggested that there may be incidental mortality as a consequence of exposing scallops to air before they are graded or measured. Joll (1988) noted the presence of trawl-induced check marks on saucer scallop shells. Such check marks indicate that trawling has a physiological effect on scallops. Saucer scallops taken by Queensland scallop trawlers can be taken in large numbers: up to 30,000 scallops per hour have been taken in optimal conditions, although average catches are more typically about 500–1,000 scallops per hour (Trainor pers. comm., Dredge 1992). The time taken to sort and grade such catches varies with catch and by-catch volume, but may be as long as an hour. Should scallops die as a consequence of being exposed to air, the incidental mor-

talidity of undersized scallops could lead to appreciable wastage and negate the value of size limits.

A short study was conducted to determine the survival of captured scallops left out of water, on the sorting tray of a trawler, over varying lengths of time. This study was designed to determine how long saucer scallops could be left out of water, in conditions similar to those encountered on a commercial trawler, before suffering mortality in excess of that observed in a control group that underwent minimum exposure to air.

METHODS

A field trial was commenced in September 1991 at an experimental site off Yeppoon (22°47'S, 151°40'E) (Fig. 1). The experiment was commenced in mid-afternoon, at which time weather conditions were sunny and warm (25°C), with wind speeds of less than 10 knots. Approximately 2,000 scallops were captured by otter trawl, with conventional paired, 11-m head rope trawls towed from a research vessel in a trawl shot of about 1.5-h duration. This is within the range of trawl shot duration normally used in the Queensland scallop fishery (Dredge and Trainor 1994). Scallops were released from the trawl nets onto a sorting tray, sorted, and treated to according to predetermined procedures.

Approximately 350 scallops were removed from the sorting tray and placed in a holding tank, through which fresh seawater was exchanged continuously. Forty-one of these were immediately measured and double tagged with individually identifiable tags (numbered Dymo-tape tags glued to shells with cyano-acrylate adhesive, after Heald 1978; Williams and Dredge 1981). A further 300 were similarly measured and tagged after being kept in the holding tank for 3 h. This group of 341 tagged scallops, all of which had been exposed to air for less than 2 min, were used as an experimental control. Tagged scallops were placed into a second deck tank of changing seawater. All remaining scallops were left on the sorting tray, simulating conditions experienced on commercial trawlers. At 30-min intervals, 41–45 scallops were taken from the sorting tray, placed in a holding tank, and then individually measured, tagged, and placed in the second holding tank. This procedure was continued for a total time of 3 h. Thus, the final

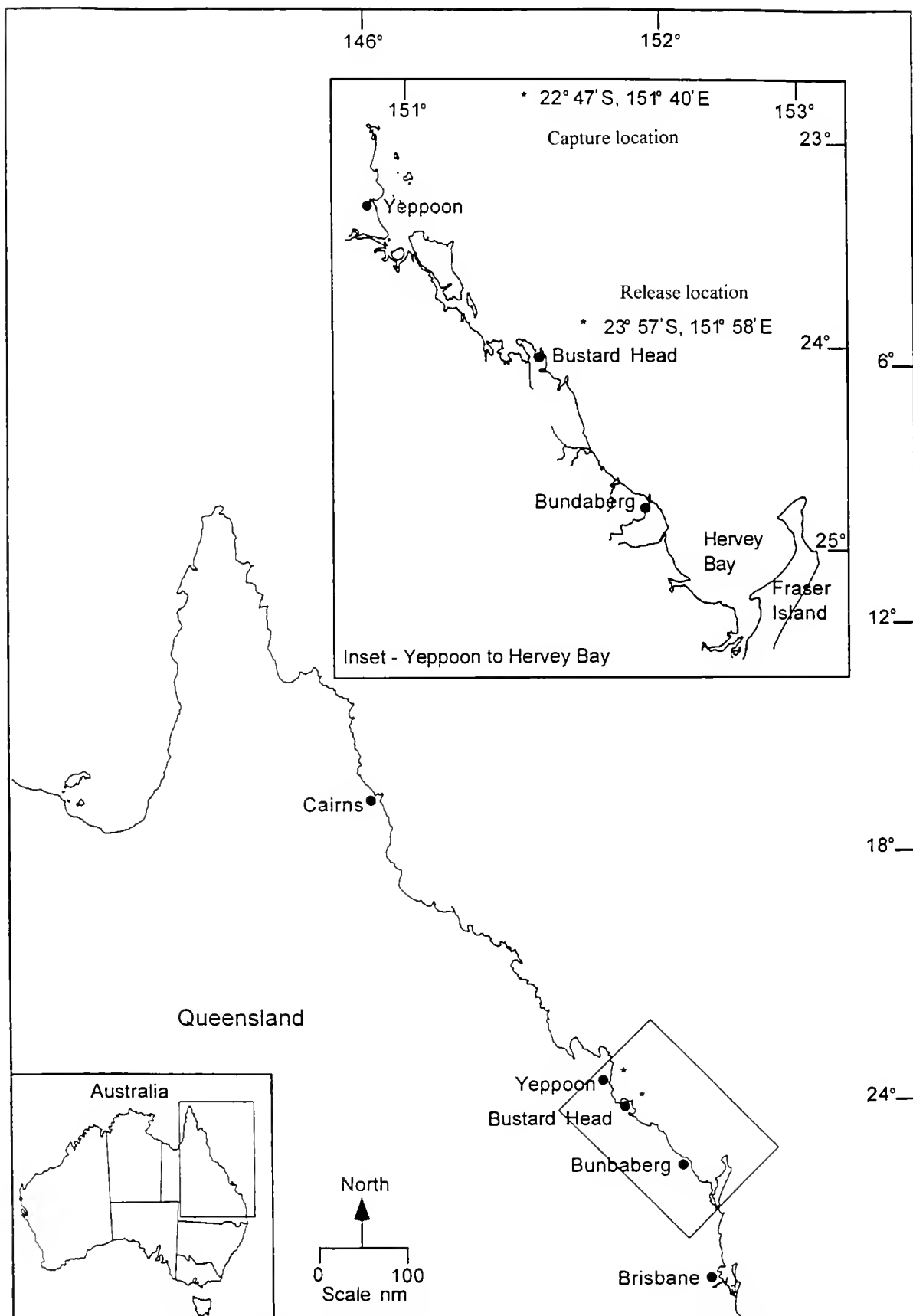


Figure 1. Location of capture and release positions off of the Queensland coast.

batch of tagged scallops had been exposed to air and prevailing weather conditions for 3 h, without being hosed, washed, or attended to in any way.

The minor variation in numbers of scallops tagged in each 30-min treatment resulted from the field staffs' ability to tag scallops at a consistent rate through the duration of the tagging operations (Table 1). A total of 597 scallops were tagged during this part of the experiment.

At the conclusion of the tagging phase, scallops were checked to find dead animals (none noted) or those that had lost a tag. Such animals were noted, but were released. Tagged scallops were released at a major fishing ground off of Bustard Head, at 23°57'S, 151°58'E, approximately 65 nautical miles south of where they were captured. Fishermen were notified of the experiment (but were not given data on the markings of scallops subjected to different exposures) and were asked to return tagged scallops to research staff.

RESULTS

One hundred fifty-one tagged scallops (25.3%) were recaptured and returned. Comparison of recapture rates as a function of size at release (Fig. 2) indicated that recapture rates did not significantly vary as a function of size at release (Kolmogorov-Smirnov test, $D_{\max} = 0.103$, $p < 0.05$, $n = 151$).

Recapture rates as a function of time exposed to air and prevailing weather conditions are given in Table 1. The recovery rate from each treatment has been compared with that from the control group (minimal exposure to air), by the use of χ^2 tests. Recovery rates between the control and other treatments were not significantly different for all treatments exposed to air and prevailing weather conditions up to and including an exposure time of 150 min, although the recovery rate for scallops exposed for 150 min was lower than that of scallops exposed for 0–120 min. Scallops that had been exposed for longer than 150 min had a significantly reduced recovery rate.

DISCUSSION

The concept of discarding undersized animals in a fishery is based on the premise that the fishery will be enhanced, either by having discards grow to a size that will increase their market value when subsequently captured or by having them contribute to an enhanced spawning stock. Saucer scallop size limits currently ap-

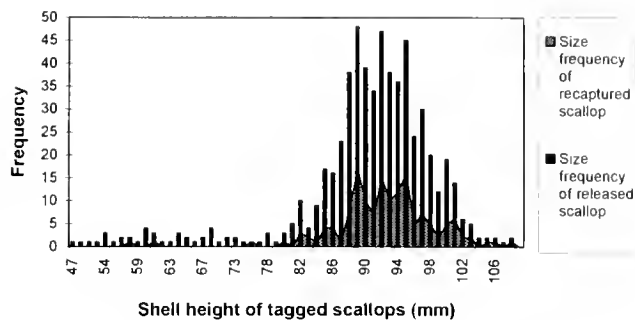


Figure 2. Size frequency composition of all tagged scallops versus size frequency composition of recaptured scallops at release.

plied to the Queensland trawl fishery are largely based around the concept of maximizing value per recruit. Implicit in this assumption is that undersized scallops survive the process of being graded and discarded.

Although there is an extensive literature on the survival of intertidal molluscs as a function of exposure to air (see, for example, McMahon 1988, Gudereley et al. 1994), few if any studies on the survival of scallops in air appear to have been undertaken or documented. Naidu and Cahill (1985) estimated tagging-induced mortality for sea scallops (*Placochelys magellanicus*) and concluded that the tagging process (which included having live, healthy animals exposed to air for short but undocumented periods) did not induce significant mortality. Brand and Murphy (1992) and Allison and Brand (1995) undertook extensive tagging programs on scallops (*Pecten maximus* and *Aequipecten opercularis*, respectively) in the Irish Sea and observed measurable mortality induced by tagging. Their studies, however, did not extend to an examination of scallop mortality rates as a function of exposure duration.

Return rates (>25% overall) give some indication of how heavily saucer scallops are being fished, at least within local areas. It is interesting to note that a replicate of this experiment, conducted some 200 nautical miles north of the release site reported here, at approximately the same time, gave tag returns of <1% (Dredge unpub. data). Such a return rate gave insufficient data to allow statistically meaningful analysis and interpretation. The importance of ensuring that tagged, sedentary animals are released in fishing grounds from which there is a high probability of recapture is emphasized by these results. The results also suggest that there has been no bias induced by the differential release or recapture of different sized scallops through the experiment. There may be the potential for field staff to unconsciously select particular size classes of scallop for tagging. If this had been the case, there may have been potential for confounding errors between size-selective mortality and mortality attributable to exposure. The data given in Figure 2 suggest that this was not the case.

The results from this study suggest that saucer scallops can withstand extended exposure to air—for upwards of 2 h—before suffering appreciable mortality. This finding is significant in the context of justifying the existing use of size limits as a yield-maximizing tool in the Queensland scallop fishery. The sorting and grading of scallop catches are not prolonged activities during normal fishing operations, with trawl catches typically being cleaned up within 30–45 min of being released onto the deck for sorting. Commercial scallop trawling is now restricted to night time as a means of reducing fishing effort from the previous 24-hour-a-day

TABLE 1.

Releases and recaptures of tagged saucer scallops.

Time Exposed (min)	Number Released	Number Recaptured	Proportion (%) Recaptured	χ^2
1	341	94	27.6	
30	41	13	31.7	0.25
60	43	16	37.2	1.44
90	45	10	22.2	0.47
120	43	13	30.2	0.11
150	42	5	11.9	3.75
180	42	0	0	11.59*
Total	597	151		

* Significant variation between return rate and that of control at 0.01 probability level.

fishery. Therefore, the stress suffered by scallops taken in the fishery is less severe than that experienced by animals in this experiment, because they are exposed when temperatures and evaporation rates are reduced. As a consequence, the utilization of size limits as a yield-maximizing technique appears to be based on sound premises.

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REPRODUCTIVE MATURITY AND SPAWNING INDUCTION IN THE CATARINA SCALLOP *ARGOPECTEN VENTRICOSUS* (= *CIRCULARIS*) (SOWERBY II, 1842)

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ABSTRACT Reproductive maturity and spawning induction were studied in the hermaphroditic catarina scallop *Argopecten ventricosus*. A closed system with seawater recirculation (3 L/min), constant temperature ($23 \pm 1^\circ\text{C}$), and salinity (37 ppt) was used. The scallops were fed 3.9×10^9 cells/animal per day of a 6:3:1 mixture of the microalgae *Isochrysis galbana*, *Chaetoceros* sp., and *Tetraselmis suecica*. Ninety-five percent of the scallops reached reproductive maturity in 27 days. For spawning induction, several methods were used. Thermostimulation combined with the addition of sexual products produced spawning in 50% of the animals and was the only method from which both gametes were obtained. Male spawning was initiated in a higher proportion than female spawning. Serotonin (5-hydroxytryptamine) was very effective, inducing sperm spawning only. The rest of the methods (electric shocks and KCl injections) failed as spawning inducers.

KEY WORDS: Reproductive maturation, spawning induction, *Argopecten ventricosus* (= *circularis*), scallops

INTRODUCTION

The catarina scallop *Argopecten ventricosus* is commercially exploited along the Pacific Coast from Mexico to Peru (Keen 1971). This species has a great potential for intensive culture, as in Mexico where some companies are successfully producing it commercially by specific methods for hatchery (Maeda-Martínez et al. 1995) and growout (Maeda-Martínez and Ormart-Castro 1995) phases. However, key factors of hatchery seed production, such as the capability to mature broodstock and to induce them to spawn throughout the year, need further attention. Sastry (1963) has suggested that gamete development to maturity in *Argopecten irradians* can be accelerated after gametogenesis has been initiated and that the rate of development to maturation is dependent on temperature. In *Mercenaria mercenaria* and *A. irradians*, gametogenesis has been induced several times in a year by controlling environmental conditions, providing the animals can recuperate from each of the postspawning activities (Loosanoff and Davis 1963, Sastry 1966). The Alligator Harbor population of *A. irradians* that spawns in late summer and autumn has been induced to maturation and stimulated to spawn throughout most of the year (Sastry 1963). Oocyte growth and spawning have been advanced by exposing animals with developing oocytes to 25°C and to 30°C (Sastry 1966). Sastry (1963) developed a reproductive maturity scale for *A. irradians*, based on the morphochromatic appearance of the gonads. Stages I to III are immature, IV is mature, and V and VI are partially spent and spent conditions. A specific five-stage scale was proposed by Villalejo-Fuerte and Ochoa-Baez (1993) for *A. ventricosus*, based on histologic observations. Some aspects of the reproductive biology of *A. ventricosus*, such as gonad index variation and gonad maturation by histologic methods, have been studied by Villalaz (1992, 1993, 1994, 1996), Villalejo-Fuerte and Ochoa-Baez (1993), and Felix-Pico et al. (1991). Artificial reproductive maturation and spawning induction in *A. ventricosus* were studied by Aviles-Quevedo and Muciño-Díaz (1988). Those authors found that adult scallops with undif-

ferentiated gonads mature in only 20 days at 18°C and 35 ppt salinity and on a diet of 4.0×10^9 to 5.0×10^9 cells/scallop per day of *Isochrysis galbana*.

The factors inducing spawning in pelecypods have been discussed in reviews by Giese (1959), Loosanoff and Davis (1963), Galtsoff (1961, 1969), Fretter and Graham (1964), Loosanoff (1954, 1971), Giese and Pearse (1974), and Sastry (1979). Temperature changes, salinity, light, mechanical shock, and chemicals have been reported to induce spawning. Temperature has been considered one of the important factors in stimulating spawning in a number of pelecypods. It has been reported that serotonin-creatinine-sulfate complex induces sperm spawning in *Argopecten irradians* (Gibbons and Castagna 1984), *Pecten albicans* (Tanaka and Murakoshi 1985), *Pecten ziczac* (Vélez et al. 1990), and *Argopecten purpuratus* (Martínez et al. 1996). In the dioecious scallop *Patinopecten yessoensis*, this neurotransmitter is effective in both males and females (Matsutani and Nomura 1982). The precise role of this complex still remains unknown. In this article, we report a method for artificial reproductive maturation for *A. ventricosus* and the efficacy of different spawning methods.

MATERIALS AND METHODS

Ripe, Stage IV (Sastry 1963) *A. ventricosus* were collected by diving at 2 to 3-m depth in Ensenada de La Paz ($24^\circ 07'\text{N}$ – $110^\circ 24'\text{W}$), Mexico, with only those measuring over 45 mm in shell length selected for the reproductive maturity and spawning induction experiments. At the time of collection, temperature and salinity were 28°C and 37 ppt. Each individual was cleaned and tagged with a plastic label tied to the dorsal auricular lobe of the shell and then left undisturbed in 1,100-L tanks with filtered seawater at $23 \pm 1^\circ\text{C}$ and 37 ppt. This procedure induced massive spawning within the following 5 h. Once spawning stopped, 300 completely spent scallops (pale brown gonads with no differentiation between testicular and ovarian regions) were selected for the reproductive conditioning experiments. Reproductive conditioning was done in a closed system with a constant seawater flow (3

L/min). Temperature and salinity remained constant at $23 \pm 1^\circ\text{C}$ and 37 ppt. The scallops were fed with a mixture of *Isochrysis galbana*, *Chaetoceros* sp., and *Tetraselmis suecica* (6:3:1). The amount of food provided was 3.9×10^9 cells/scallop per day. Every 4 days, gonadal condition was visually checked. When reproductive maturity was again reached, the following spawning induction methods were tested in 20 individuals for each method: sudden 12°C thermal shock ($18\text{--}30^\circ\text{C}$); fast thermal change from 18 to 30°C over 4 min ($3^\circ\text{C}/\text{min}$); gradual thermal change over 12 min ($1^\circ\text{C}/\text{min}$); gradual thermal change ($1^\circ\text{C}/\text{min}$) with sexual product addition (Loosanoff and Davis 1963); 0.025, 0.25, and 2.5 mM intragonadal serotonin (5-hydroxytryptamine) injections (Tanaka and Murakoshi 1985); 0.5, 1.0, and 2.0 mM intragonadal KCl injections (Young 1945); and electric shocks (20 V for 1 sec) (Iwata 1951). Thermal shock experiments were done in 70-L tanks. Initially, each tank contained seawater at the same temperature and salinity as in the reproductive maturation experiment. Then, warm seawater was siphoned into the tanks at a rate that produced the desired temperature change until 30°C was reached. Spawning response was considered fast, medium, or slow if gamete release began in <3 h, from 3 to 5 h, or >5 h from the stimulus application.

RESULTS

Tagging techniques allow an exact observation of the gonad behavior and good control to avoid using the same animal in different experiments, although there is no damage. In Figure 1, the development of the *A. ventricosus* gonad is shown during the experiment. In Stage I or the indifferent stage, gonadal tissue was transparent and it was not possible to distinguish the portion corresponding to each sex. On the second conditioning day, a few follicles of the gonads on 5% of the animals had developed spermatogonia and oogonia, as seen by microscopic examination (Stage II). Between days 7 and 9, 85% of the individuals were Stage II. After day 18, 85% of the animals were Stage III, char-

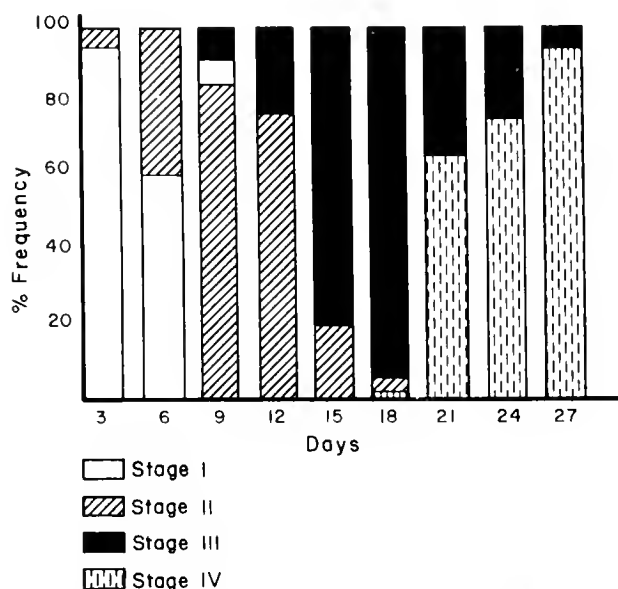


Figure 1. Temporal change in gonad maturation of 45-mm-shell-length *A. ventricosus*, fed with 3.9×10^9 cells/scallop per day of a mixture of *I. galbana*, *Chaetoceros* sp., and *T. suecica* (6:3:1) at $23 \pm 1^\circ\text{C}$ and 37 ppt salinity. $n = 300$ scallops.

TABLE 1.

Spawning response of ripe (Stage IV; Sastry 1963) *A. ventricosus* to different stimuli ($n = 20$ individuals per treatment).

Method	Response (% of Group Spawning)			% Initial Spawn	
	Fast <3 h	Medium 3–5 h	Slow >5 h	Female	Male
Sudden thermal shock, $18\text{--}30^\circ\text{C}$ in 1 sec		20		25	75
Fast thermal change, $3^\circ\text{C}/\text{min}$ increase ($18\text{--}30^\circ$)			10	50	50
Gradual thermal change, $1^\circ\text{C}/\text{min}$ increase ($18\text{--}30^\circ\text{C}$)		30		35	65
Gradual thermal change (as above) plus gamete addition		50		40	60
Average % spawn using thermal stimuli				37.5	62.5
Serotonin injection (0.025, 0.25, or 2.5 mM)	100				100

acterized by a uniform pigmentation of the cream-colored testicle and the orange ovary. Gonadal volume was considerably increased at this time. Stage IV, the mature stage, showed brilliant colors in both gonadal portions, dark cream for male and red-orange for female. Pigmentation was very smooth, and gonadal volume increased as compared with somatic tissue. On day 27, 95% of the animals were in Stage IV.

Table 1 presents the results of the spawning induction experiments. Sudden thermal shock ($18\text{--}30^\circ\text{C}$) induced spawning in only 20% of the individuals. With this treatment, a medium response was obtained (between 3 and 5 hours from the stimulus application), and in most cases (75%), sperm was released first. In the fast and gradual thermal change treatments, (3 and $1^\circ\text{C}/\text{min}$ from 18 to 30°C), only 10 and 30% of the individuals spawned. Response in these treatments was slow and medium, respectively. When the latter treatment was applied with the addition of sexual products from another scallop, response improved to 50%. Time of response remained at a medium level. An average of female and male initial-spawn percentages from our thermal treatments (Table 1) showed that only 37% of spawning began with ova release whereas 63% were male spawnings. Those scallops that spawned continued to do so, switching from one sexual product to the other, following a random pattern. Serotonin induced sperm spawning in 100% of scallops injected. For the three concentrations tested, the response was the same and fast. Sperm was released 9 ± 1 min after injection. However, this method seemed to be very stressful because the animals opened and closed their valves violently. Because of this movement, there was even a loss of gill fragments. Ejaculation ceased at about the third hour after injection. Ova release after serotonin injections was not observed during this time. There was no response to electric discharge or intragonadal KCl injection at any of the concentrations tested.

DISCUSSION

In the *Pectinidae*, visual examination of the gonad is a direct method of distinguishing the sex and maturity stage on the basis of morphochromatic appearance. Our results show that Sastry's scale developed for *A. irradians* could be applied in *A. ventricosus*. In a production hatchery, the morphochromatic method is a fast and reliable alternative in selecting the broodstock for spawning.

The reproductive maturity method described in this article produces ripe broodstock in only 27 days at 25°C and 3.9×10^9 cells/scallop per day of a mixture of microalgae. It is not known, however, if this treatment accelerates reproductive maturation when compared with the wild. Aviles-Quevedo and Muciño-Díaz (1988) achieved full maturation in this species in less time (20 days), in colder (18°C) conditions, and with a higher ration (4.0×10^9 to 5.0×10^9 cells/scallop per day). The 7-day difference with our results could be caused by a lack of precision in the selection of the broodstock by Aviles-Quevedo and Muciño-Díaz (1988). Those authors used undifferentiated scallops, meaning that Stage II animals could have been used. With the naked eye, it is not possible to distinguish the portion corresponding to each sex in this stage, but maturation is already well advanced. In contrast, in our experiments, the whole cycle from spawning to spawning was considered. If those authors actually matured Stage VI scallops, two alternatives could explain the 7-day difference: (a) scope for activity measurements indicate that in *A. ventricosus*, there is higher energy available for growth and reproduction at 19°C than at 25°C (M. T. Sicard pers. comm.) and (b) Aviles-Quevedo and Muciño-Díaz used a higher food ration, which probably promoted faster gonad maturation. In a similar scallop (*A. irradians*), maturity under laboratory conditions is reached in 26–30 days at 18°C (Castagna and Duggan 1971) and in 35 days at $29 \pm 1.0^\circ\text{C}$ (Sastry 1963), when held in running raw marine water.

Thermostimulation is one of the common methods to induce spawning in molluscan species (Loosanoff and Davis 1963). Of the methods used for spawning induction, temperature shock seems to be the alternative to obtain sperm and ova from ripe *A.*

ventricosus. The efficiency of this inducer is improved if sexual products from another scallop is added to the spawning tank. Enhanced effectiveness of thermal stimulation combined with the addition of gametes of the opposite sex has been reported for a number of species (Loosanoff and Davis 1963, Bayne 1965). Wada (1954) has reported that the addition of an egg water suspension or a sperm suspension stimulates spawning in *Tridacna*.

Even if our temperature treatments seem to be stressful, there appears to be no negative effect on development because the resultant larvae were cultured in the laboratory. Another spawning alternative in this work proved to be the combination of handling (mechanical shock) with a temperature change. Although this method was not evaluated, it produced an unwanted spawning shortly after scallop collection. In *A. ventricosus*, male spawning was initiated in a higher proportion than female spawning. This also occurs in *A. irradians*, where spermatozoa are released more readily than are ova (Sastry 1966). No explanation was found for this.

In *A. ventricosus*, serotonin is a very effective inducer of sperm spawning, as it is in *A. irradians* (Gibbons and Castagna 1984), *P. albicans* (Tanaka and Murakoshi 1985), *P. ziczac* (Vélez et al. 1990), and *A. purpuratus* (Martínez et al. 1996). Serotonin, however, fails to induce ova spawning in these hermaphroditic species, whereas in a dioecious species such as *P. yessoensis* (Matsutani and Nomura 1982), it induces spawning in females. Martínez et al. (1996) have recently found that injections of dopamine and prostaglandin E_2 , with a 30-min lapse between them, successfully induced ova and sperm spawning in *A. purpuratus*. This is a promising alternative to be tested in *A. ventricosus* and other hermaphroditic scallops.

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PRIMARY AND SECONDARY SETTLEMENT BY THE GREENSHELL MUSSEL *PERNA CANALICULUS*

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ABSTRACT The settlement and postsettlement dispersal behavior of the mussel *Perna canaliculus* was investigated in laboratory- and field-based experiments to determine the role of primary and secondary settlement in its early life history. Field survey data showed size-specific patterns of mussel residency on a variety of rocky shore floral and faunal substrata. Primary settlement (<0.5 mm), both in the laboratory and in the field, was largely on the hydroid *Amphisbetia bispinosa* and the turfing algae *Corallina officinalis*, *Champia laingii*, and *Laurencia thyrsoifera*. In the field, postlarvae between 0.5 and 5.5 mm occurred mostly on the algae *C. officinalis*, *C. laingii*, *L. thyrsoifera*, *Melanthalia absissa*, *Pterocladia lucida*, *Gigartina cranwellae*, and *Gigartina alveata*. Juvenile and adult mussels (>5.5 mm) were resident predominantly on *P. lucida*, *G. alveata*, and *Pachymenia himantophora*. Size-frequency data from established mussel beds indicated low levels of primary settlement, with the majority of recruitment coming from secondary settlement of larger individuals. Recruitment patterns were consistent with Bayne's primary-secondary settlement model. Substrata deployed onto and near the shore for 21 days recruited both a primary settlement cohort and a secondary settlement cohort of mussels too large to have originated from primary settlement. Differential residency patterns in the field and settlement/recruitment experiments suggested a change in substratum preference by juveniles as a function of size and age. It is suggested that mucus drifting was the likely means of movement for young postlarvae among habitats. Mucus-drifting experiments demonstrated that postlarvae <6 mm in length were able to slow their rate of descent in the water column to 30% of the passive sinking speed using long mucus threads. The size at which *P. canaliculus* were able to use this method of dispersal greatly exceeds that seen in *Mytilus edulis*.

KEY WORDS: Mussel, *Perna canaliculus*, settlement, dispersal, mucus drifting, byssopelagic migration

INTRODUCTION

Many marine mussel species settle preferentially on fine filamentous substrata and seldom in the main adult bed (de Blok and Geelen 1958, Bayne 1964, Seed 1969, King et al. 1989, King et al. 1990, Cáceres-Martínez et al. 1993). Subsequent dispersal of postlarvae is thought to be the major means of recruitment of juvenile mussels into adult beds. Juveniles of *Mytilus edulis*, once detached from the site of primary settlement, may "drift" in the sea, suspended under a thread of mucus, a behavior termed byssopelagic migration (Sigurdsson 1976, de Blok and Tan-Mass 1977, Lane et al. 1985). The mussel is thus able to move over significant distances and then reattach at some different site. This detachment and reattachment behavior may occur numerous times before recruitment into the adult bed occurs. Bayne (1964) first linked the depletion of juvenile mussels attached to seaweed with the concurrent recruitment of these same size classes into the adult bed, a pattern later recognized by others (Dare 1976, Seed 1976). It has been suggested that this primary settlement–dispersal–secondary settlement and later recruitment into the adult bed represent an adaptive trait to remove settlement of the larvae from unfavorable conditions within the adult bed (Bayne 1964, Lane et al. 1985.) Evidence for primary settlement directly into the adult bed has raised some controversy as to the general applicability of the primary-secondary settlement model (Petersen 1984a, Petersen 1984b, McGrath et al. 1988, King et al. 1990, Lasiak and Barnard 1995, Cáceres-Martínez et al. 1994).

Perna canaliculus or the Greenshell mussel is a common bivalve in New Zealand, growing naturally on lower intertidal and sublittoral coastal shores and on sandy bottoms in deeper water

(Paine 1971). Greenshell mussels are intensively farmed in parts of New Zealand, making this shellfish the most important aquaculture species in the country. The larvae of *P. canaliculus* settle in large numbers on a variety of coastal and drift substrata, such as seaweeds and hydroids (Hickman 1976). However, abundant primary settlement on commercial spat catching ropes is often followed by virtually complete loss over the following weeks, causing spat supply problems for the Greenshell mussel industry. Spat loss may, in part, be due to juvenile dispersal. This study aimed to identify patterns of settlement and substratum preferences in *P. canaliculus*, as well as to assess the role of byssopelagic dispersal and the applicability of the primary-secondary settlement model in the recruitment of this species.

MATERIALS AND METHODS

Mussel Habitat Survey

Two study sites were chosen at either end of Piha beach on the West Coast of the North Island, New Zealand (36°S, 174°E). This coast is highly exposed to almost continuous wave action and has a tidal range of approximately 3 m. The volcanic conglomerate rocky shore supports large beds of adult *P. canaliculus*, extending from approximately 2 m above chart datum to a lower level of about 0.5 m below chart datum. The shoreline is inhabited by a large variety of algal and hydroid substrata on which young mussels are abundant. Nine species of substratum were chosen for the survey: *Corallina officinalis* (COR), *Champia laingii* (CHA), *Melanthalia absissa* (MEL), *Pterocladia lucida* (PTE), *Gigartina alveata* (G.A.), *Gigartina cranwellae* (G.C.), *Pachymenia himantophora* (PAC), *Laurencia thyrsoifera* (L.T.), and *Amphisbetia bispinosa* (AMP). All species occurred at both sites, except for L.T., which was found at Site 1 only, and AMP, which was found at Site 2 only. Three replicate whole-algal samples of each substratum

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species were collected by hand, by separating the substrata hold-fast from the rock to which they were attached within an area between 0.1 and 0.4 m above chart datum. Samples were collected during spring tide over 10 sample collections spanning the January to November period of 1993. In addition to the above substrata, three circular 15-cm² core samples of adult mussel bed were collected by scraping off the animals within the core. Individual samples were placed in plastic bags and frozen for later analysis.

Samples were thawed, and small mussels were removed by vigorous agitation in a domestic bleach solution [1.2% (v/v) hypochlorite], which removed the majority of smaller animals. Any remaining individuals were removed with tweezers. Mussels removed from substratum samples were recorded on video under appropriate magnification, and the resultant image was captured by JAVA image analysis software (Jandel Scientific). Maximum mussel lengths from umbo to ventral margin were then obtained, and length measurements were arranged into size-frequency data.

Size-frequency data were further classified into three groups representing different life history stages, as identified in the dispersal experiments. The first category was the "settlement cohort": mussels of ≤ 0.4999 mm in length. *P. canaliculus* larvae typically settle at sizes between 250 and 300 μ m (Redfearn et al. 1986, King et al. 1989, Cáceres-Martínez et al. 1993). The "dispersal cohort" contained animals that had the ability to disperse by mucus drifting. These animals were within the size range of 0.5–5.4999 mm in length. The third category was the "stable cohort": animals >5 mm, representing the proportion of the population that was unable to use mucus drifting as a means of dispersal. All size-frequency data were expressed as proportions and normalized by arcsine square root transformation before statistical analyses were performed. Data were analyzed using single-factor Kruskal-Wallis analysis of variance (at $\alpha = 0.05$, $k = 10$, $n = 30$), after Bartlett's tests for homogeneity of variance (at $\alpha = 0.05$) identified strong heteroscedasticity in the data. *A posteriori* Tukey-type Nemenyi multicomparison testing at $\alpha = 0.05$ was performed for all substrata at each size class for both sites (Zar 1996). This test was used to identify significant differences in the proportional mussel occupancy between substrata in each of the three size cohorts.

Transplant Experiment

Samples of substrata were collected at low tide from the rocky shore at Site 2. The substrata used were AMP, COR, PTE, MEL, and PAC. Samples were removed with a cold chisel, together with the rock to which they were attached, and transported to the laboratory. Mussels were removed from the substrata with tweezers under magnification to ensure that no mussels remained. The rocks to which the substrata were attached were then fixed to tiles (200 \times 80 mm) with a fast-curing cement and transported back to the field site. Tiles were cemented directly onto the rocky shore or attached to steel frames positioned approximately 2 m away from the rocky shore in adjacent sand banks. The shore-based and frame-based transplants were composed of 6 and 15 replicate samples, respectively, and were located at 0.2 m above chart datum. After a period of 21 days, the remaining substrata were collected and returned to the laboratory. Because of storm conditions, some samples were lost from both sites. All mussels on the substrata were removed and measured. These measurements were then transformed into size-frequency data for each substratum type as described above. Proportional occupancy in the size classes of

resident mussels that could have primarily settled on the substrata (<0.5 – 1.5 mm) and the proportion that colonized via dispersal (size class >1.5 mm) for each sample were normalized by the arcsine square root transformation. Single-factor analysis of variance ($\alpha = 0.05$) for each size range (settlement and recolonization) was performed for both transplant types (shore and remote). Tukey multiple comparison ($\alpha = 0.05$) was used to identify significant differences among the substrata in both the settlement and the recolonization size ranges for the two transplant types.

Sinking Velocity

A 1.5-m-tall vertical glass column of 2-cm internal diameter, based on the design of Lane et al. (1985), was used to determine the sinking rates of freefalling and mucus-drifting juvenile *P. canaliculus*. The column was marked at 5-cm intervals and filled with freshly filtered (10- μ m-pore-size filter) seawater at ambient temperatures ($16 \pm 1^\circ\text{C}$). Individual mussels collected off of seaweed from the experimental site were placed in the top of the column and allowed to descend freely. The time interval between consecutive 5-cm intervals was measured with a computer-based timer, and the rate of descent was calculated. The experiment used individuals between 1.5 and 2 mm in length, both live and dead (fixed in 5% formalin). These experiments demonstrated the ability of mussels to reduce sinking rate but were not suitable to describe the slowest sinking velocities of mucus drifters. To measure the slowest sinking velocity, a variable flow chamber was constructed such that prolonged mucus production was possible and sinking rates could stabilize. A longitudinal half-section of 65-mm polyvinyl chloride (PVC) pipe was attached to the inside of a tall (850-mm) glass aquarium. Vertical water flow up the pipe was controlled by varying the water supply through a diffuser at the base of the column. Fine chalk powder could be added to the column, making the invisible mucus thread produced by mussels visible. Animals in the size range of 0.6–8 mm in length were introduced into the top of the flow chamber and allowed to fall against the water flow. As mucus secretion progressed, water velocity could be adjusted to prevent the animal from being washed out of the chamber. Once the sinking rate of the mussels stabilized, the water flow was stopped and three to five replicate measurements of slowest sinking velocity for each individual were taken. The experiment compared both actively mucus-drifting and non-active live mussels.

Settlement Preference in the Laboratory

Sexually mature mussels were collected from the field site in midspring and transported to the laboratory, where they were immediately induced to spawn using temperature shock ($+8^\circ\text{C}$ from ambient) in conjunction with stripped sperm. Spawned eggs were collected and suspended in 200-L tanks with 10 filtered (10- μ m-pore-size filter) seawater at 21 – 23°C , into which a small amount of sperm was added. Resultant larvae were fed on *Isochrysis galbana* and *Pavlova lutheri* for the first 14 days. From Day 15 onward, *Chaetoceros gracilis* and *Chaetoceros calcitrans* were added to the diet to give a final algal concentration of approximately 25–35 cells/ μL . Larvae were competent to settle after 25 days. Larvae were placed in an experimental environment containing four randomly placed replicates of five mussel-free substratum types, among which they could move freely, and were allowed to settle over 4 days. The apparatus consisted of 20 PVC tubes of 5 cm in diameter and 3 cm in length, covered on the bottom end with

160- μ m pore size mesh. The tubes were set with the top end flush with the bottom of a rectangular PVC open tray. The whole apparatus was suspended in a 250-L tank (Fig. 1). The apparatus received water input via two water uplifters at approximately 100 mL/min ($\approx 15\%$ total volume). At the end of the experiment, the substrata were gently removed and all settled and metamorphosed mussels were counted for each substratum replicate. The substrata offered were hydroid AMP, COR, *Laurencia botryoides* (L.B.), MEL, and PAC. Adult mussels were not offered as a substrata choice because observations showed that adult ventilation often binds larvae in pseudofeces, usually killing them. Settlement preference was calculated in two ways: first, in terms of mussel numbers per gram wet weight, and second, as specific surface area (cm^2/gm wet weight) of the substratum offered. Surface area was estimated using JAVA image analysis measurements of a known wet weight of substrata and replicated until variance was $\leq 15\%$ of the mean.

RESULTS

Mussel Residency Survey

All of the substrata collected were used by mussels as sites of attachment, and settlement occurred throughout the year, with a peak period in the spring to summer season. Significant differences ($p < 0.001$) in substratum preference were observed among mussels of different size classes (Table 1). Three general patterns of occupancy among the different substrata could be distinguished: (1) AMP, L.T., CHA, and COR had high proportions of primary settlers, many residents in the "dispersal" cohort, and an extremely low proportion of "stable" mussels. (2) G.A., G.C., and PTE had an intermediate pattern with substantial proportions in each cohort. (3) MEL, PAC, and mussel bed had a low proportion of settlers and increasing proportions in the "dispersal" cohort, with the majority of occupants in the "stable" cohort.

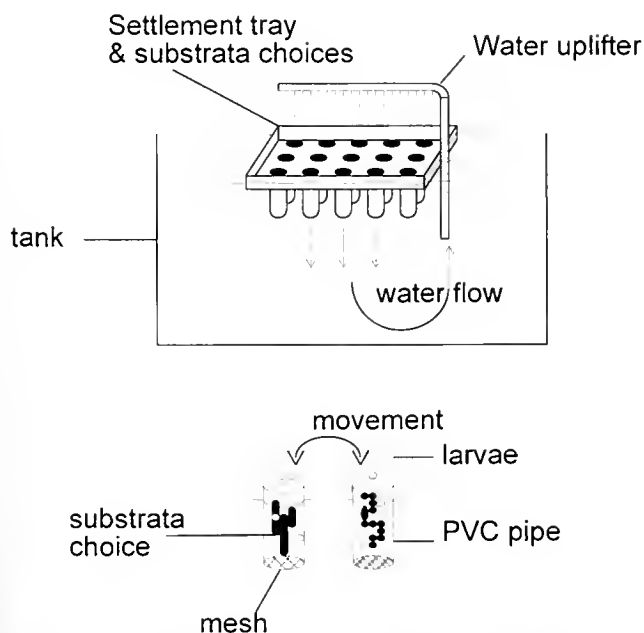


Figure 1. Diagram showing design of the settlement preference apparatus. Substrata were contained within PVC tubes; larvae were able to move freely between substrata choices but were contained within the tray. The apparatus was suspended in a 250-L tank and received water and nutrient flow via a water uplifter.

Transplant Experiment

Growth of juvenile *P. canaliculus* at 22°C in the laboratory indicated a growth rate of $\approx 21 \mu\text{m}/\text{day}$ (S. Buchanan, unpubl. data), results similar to those of Bayne (1965), who reported a growth rate of $25 \mu\text{m}/\text{day}$ in *M. edulis*. At this growth rate, any primary settlers (at $300 \mu\text{m}$) on the substrata presented in the transplant experiment should not have exceeded $740\text{--}825 \mu\text{m}$ in length after 21 days. The cutoff between primary settlers and potential migrant residents on the transplant substrata was extended to 1.5 mm (between the second and third size classes) to ensure that no primary settlers were misrepresented as postdispersal residents. The transplant experiment demonstrated that postlarval mussels colonized the new substrata. For both the shore transplants and the remote transplants, approximately 55% of the mussels on the transplants were too large ($>1.5 \text{ mm}$) to have originated from the primary settlement and growth within the 21-day experimental period during which the test substrata were deployed (Fig. 2). A significantly differential pattern of secondary settlement specificity was found that reflected results of field survey and laboratory settlement experiments. At both transplant sites, differences among the substrata in both the potential primary settler size range and the recolonization size range were significant. On the finely branched substrata COR (settlement: shore, 86%; remote, 89%) and AMP (settlement: shore, 84%; remote, 87%), the majority of recruits were within a size range that could have come via primary settlement, with few animals having been recruited by the secondary settlement pathway. In contrast, the coarsely branched substrata PTE (settlement: shore, 49%; remote, 59%), MEL (settlement: shore, 36%; remote, 36%), and PAC (settlement: shore, 0%; remote, 20%) recruited a greater proportion of resident mussels via a secondary settlement pathway than from primary settlement. These results indicated that movement among substrata, rather than differential mortality alone, was a major source of differential distribution of juvenile *P. canaliculus* on various substrata.

Sinking Velocity

Over a distance of 1.5 m, many sinking mussels were able to slow their rate of descent from 5 to around 2 cm s^{-1} (Fig. 3). These animals produced mucus threads from the extended pedal organ through the course of their descent. In comparison, dead or inactive animals maintained a sinking rate close to 5 cm s^{-1} throughout. Results from the variable flow chamber demonstrated the effectiveness of the mucus production as a means to reduce the sinking rate (Fig. 4). In all size classes, a velocity reduction to at least 50% of the non-mucus-drifting speed occurred once mucus production reached its peak and slowest sinking rate was established. Slowest sinking rate was highly correlated with mussel size for both active and nonactive animals. In smaller animals ($<3 \text{ mm}$), a greater reduction to 30% of maximum velocity was usual. The largest animal to produce mucus and effectively reduce its sinking rate was 6 mm in length. The mucus thread, once marked with chalk powder, showed that although mucus was secreted as a fine thread, it often became entwined, folded on itself in the current, and appeared much like a tangled parachute. The mucus thread often exceeded 20–25 cm in length, over 100 times the length of the mussel itself.

Settlement "Preference" in the Laboratory

Various substrata attracted significantly different levels of mussel settlement [Tukey's HSD performed on $\log(\chi)$ transformed data]. Larvae showed a significantly higher settlement on the

TABLE 1.

Mean occupancy of *P. canaliculus* postlarvae in settlement, dispersal, and stable size classes on various substrata at Sites 1 and 2.

Site 1							Site 2						
Size Class	Settlement		Dispersal		Stable		Size Class	Settlement		Dispersal		Stable	
Kruskal-Wallis Statistic & probability	51		50		98		Kruskal-Wallis Statistic & probability	114		78		172	
	0.001		0.001		0.001			0.001		0.001		0.001	
Sub-stratum	Mean Occupancy Proportion	Nemenyi Test Ranking	Mean Occupancy Proportion	Nemenyi Test Ranking	Mean Occupancy Proportion	Nemenyi Test Ranking	Sub-stratum	Mean Occupancy Proportion	Nemenyi Test Ranking	Mean Occupancy Proportion	Nemenyi Test Ranking	Mean Occupancy Proportion	Nemenyi Test Ranking
COR	0.24	A	0.72	ABC	0.04	DE	AMP	0.47	A	0.51	B	0.02	DE
G.A.	0.17	B	0.50	CD	0.32	BC	CHA	0.34	AB	0.54	B	0.11	DE
L.T.	0.17	AB	0.68	AB	0.16	CD	COR	0.30	BC	0.67	AB	0.03	EF
CHA	0.11	AB	0.72	A	0.17	CD	PTE	0.20	BC	0.52	B	0.28	BC
G.C.	0.11	B	0.74	AB	0.15	CD	G.C.	0.14	D	0.66	AB	0.20	CD
MEL	0.08	B	0.60	ABC	0.32	B	G.A.	0.13	CD	0.29	C	0.58	A
PTE	0.03	B	0.61	BC	0.36	B	MEL	0.09	D	0.77	A	0.14	CD
MUS	0.03	B	0.32	DE	0.64	A	MUS	0.02	DE	0.27	C	0.71	A
PAC	0.00	C	0.46	E	0.54	C	PAC	0.02	E	0.36	C	0.62	AB

Mean occupancy frequency of resident mussels in the settlement, dispersal, and stable size classes for each substratum over the survey period for Sites 1 and 2. Kruskal-Wallis single-factor analysis of variance statistics are shown. All size classes produced highly significant results ($p < 0.001$ at $\alpha = 0.05$) at both sites. Tukey type *a posteriori* Nemenyi test results are represented by alphabetical characters. Substrata marked with the same character are not significantly different from one another. Substratum: *Corallina officinalis* (COR), *Champia laingii* (CHA), *Melanthalia absissa* (MEL), *Pterocladia lucida* (PTE), *Gigartina alveta* (G.A.), *Gigartina cranwellae* (G.C.), *Pachymenia humantophora* (PAC), *Laurencia thyrsefera* (L.T.), *Amphibetia hispidosa* (AMP), and adult mussel bed (MUS).

finely branched substrata AMP and COR in comparison to the substrata MEL and PAC, which were the most coarsely branched. *L. botryoides* attracted an intermediate settlement density (see Fig. 5). This relationship was seen for settlement expressed as a function of both substratum weight and surface area. On the finely branched substrata, postlarvae were found attached both to branches and at bifurcations within the branching structure.

DISCUSSION

P. canaliculus is able to disperse using the mucus-drifting mechanism also seen in other species of mussels (Sigurdsson 1976, de Blok and Tan-Mass 1977, Lane et al. 1985). Juveniles at lengths of <1 mm exhibited the slowest sinking velocities, <0.5 cm s^{-1} , results similar to those of Lane et al. (1985), who recorded velocities in the order of 0.3 down to 0.03 cm s^{-1} for 500- to 700- μ m *M. edulis*. Although Lane et al. (1985) did collect data for *M. edulis* indicating near-slowest sinking velocity at lengths ≈ 1.7 mm, the maximal size at which the postlarvae of this species are able to mucus drift is unclear. Data presented here demonstrated a maximal size limit of 5–6 mm for mucus drifting of *P. canaliculus* postlarvae, a size significantly larger than the range of ≈ 1 –2.5 mm suggested for *M. edulis* (Bayne 1964, de Blok and Tan-Mass 1977, Lane et al. 1985, King et al. 1990). It has been suggested that the upper size limit for mucus drifting is set more by anatomical changes of pedal glands (Lane et al. 1982) rather than by an inability to cut byssus anchors (Board 1983). If true, this would suggest a prolonged retention of these anatomical features in *P. canaliculus*.

In the wave surges common at the experimental sites used in the survey, upwelling velocities in excess of typical terminal sinking velocities would be expected to be common. Additionally, small air bubbles generated in the surf and bound to mucus would give significant buoyancy to mucus-drifting mussels in this environment. Un-

fortunately, observations of mucus drifting in the field are nearly impossible, and there are no reported observations of such activity in mussels. Postlarvae that are at least neutrally buoyant as the result of mucus may drift over considerable distances, certainly in the order of meters, from the site of original detachment. Observations in this study, of mussels crawling up mucus threads that had attached to the overflow of the variable flow chamber, suggest that mucus thread dispersal serves not only as a means of dispersal but also as an extension of the body that can come in contact with attachment sites, also described by de Blok and Tan-Mass (1977). The transplant experiments demonstrated that resettlement occurred at the experimental site. Dispersal size class mussels on the shore transplants may have colonized these substrata using byssus reattachment or foot walking; however, this mode of movement was not available at the remote transplant destination where resuspension of the postlarvae is necessary. Postdispersal residents on the remote transplants, located adjacent to, but not continuous with, the rocky shore could have only arrived there through transport in the water column, most likely via mucus drifting. The data demonstrated the important contribution that secondary settlement made to the resident mussel population on these experimental substrata, particularly to the thicker branched species PAC (70%), MEL (60%), and PTE (50%) (Fig. 5). In contrast, mussels arriving as primary settlers dominated the population on finer substrata such as COR (85%) and AMP (85%).

It was evident from the field survey that a large range of substrata were accepted as settlement sites for *P. canaliculus* pediveligers; however, there was a higher settlement rate on the filamentous substrata COR, CHA, *L. botryoides*, L.T., and the hydroid AMP in the field (Table 1). This pattern was supported by results of laboratory experiments (Fig. 5). These filamentous species play an important role in community structuring and can be viewed as focal points of intense *P. canaliculus* settlement. Low settlement specificity to substrata of similar form suggests that the attraction

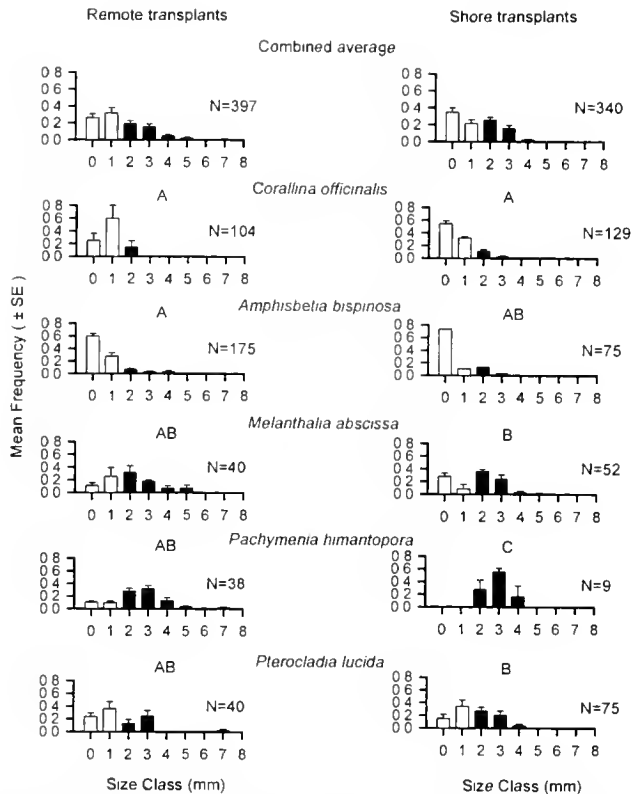


Figure 2. Average frequency as a proportion of the total (\pm SE) of resident mussels at eight size classes on five substrata from shore- and remote-based transplants after a period of 21 days. Open columns represent size classes of residents that may have been primary settlers; filled columns represent the size classes of mussels that could not have originated from primary settlement within the 21 days of deployment of test substrata. Substrata on which the proportion of recolonization occupancy was not significantly different at $\alpha = 0.05$, analyzed by Tukey's HSD, have the same alphabetical character above the histogram.

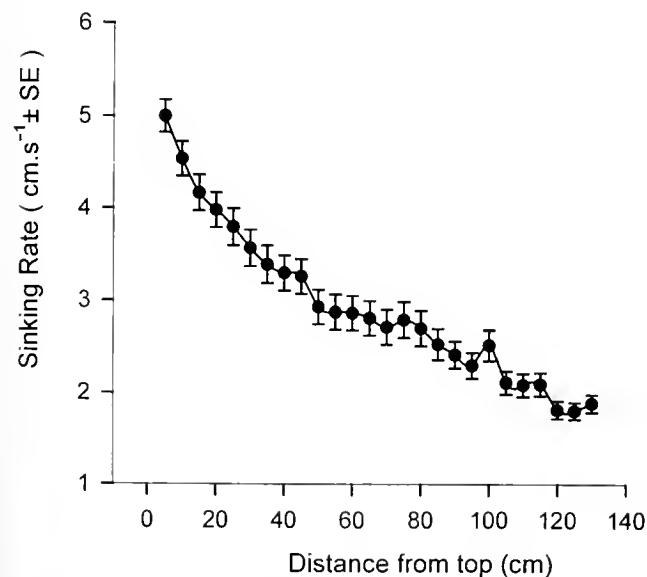


Figure 3. Change in average sinking velocity \pm SE per 5-cm interval of nine active mucus-drifting juvenile *P. canaliculus* (1.5–2 mm) during descent in a 1.5-m glass column.

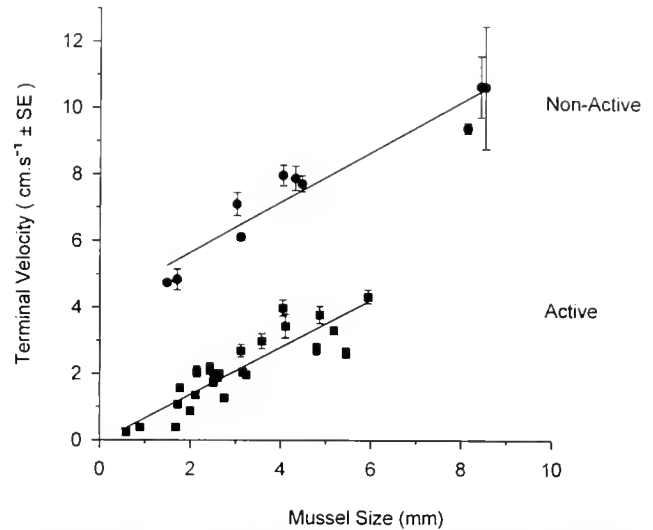


Figure 4. Average sinking velocity \pm SE of non-mucus-drifting *P. canaliculus* (●) and active mucus-drifting *P. canaliculus* (■), determined in a variable flow chamber. Non-mucus drifters, $R^2 = 0.926$; active mucus drifters, $R^2 = 0.809$.

of mussel pediveligers to a particular settlement substratum is related more to the general morphology than to chemical composition (Seed 1976), although chemical (Cooper 1982, Eyster and Pechenik 1987), biological (Falmange 1982), and hydrodynamic processes (Taylor and Beattie 1984, Martel et al. 1994) may also play roles in the selection of settlement sites.

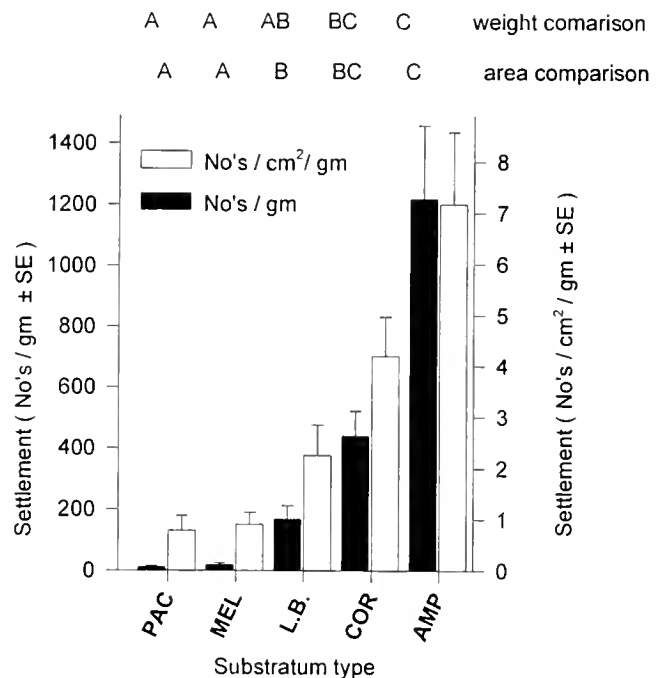


Figure 5. Primary settlement preference of *P. canaliculus* larvae in the laboratory. Average numbers \pm SE per gram of substrata (black bars) and per cm^2 of substrata (white bars). Substrata on which settlement preference was not significantly different at $\alpha = 0.05$, analyzed by Tukey's HSD, have the same alphabetical character above the bar, for numbers \pm SE per gram of substrata (weight comparison) and for numbers \pm SE per cm^2 of substrata (area comparison). Substratum are as given for Table 1.

Size-specific distribution patterns observed in the field survey can largely be explained by an active ontogenetic change in post-larval substratum preference with increasing size (and age) facilitated by byssopelagic dispersal. Differential mortality particular to different substratum types may also account for these patterns; however, the behavioral, demographic, and experimental evidence showed that significant levels of recolonization of natural substrata occurred by >1.5-mm-plus juveniles (even on substrata discontinuous with the normal habitat), indicating that dispersal was likely to be the more important of these two processes on many substrata. Substrata such as AMP appeared to have rapidly lost mussel residents of more than a few millimeters in length, whereas others such as PAC and the adult mussel bed appeared to receive individuals by means other than primary settlement. In contrast, in other substrata such as PTE, primary settlement alone could account for the mussels resident.

The comparatively low levels of primary settlement in the main adult bed suggested that secondary settlement forms the most significant mode of recruitment there. The results appear largely consistent with Bayne's (1964) primary-secondary settlement model, in which primary settlement and growth are followed by a dispersal phase that accounts for the juvenile recruitment into the adult bed. This infers that, as an adaptive mechanism to avoid

competition with adults, veligers choose not to settle into the adult bed, despite byssal threads providing ample suitable filamentous substrata there (Ester and Pechenick 1987). High survival of primary settling juveniles on filamentous material outside the adult bed, leading to an accumulation of dispersed animals, coupled with low levels of survival of primary settlers within the adult bed, may also account for the common pattern that is the basis of the primary-secondary settlement model. Bayne (1964) observed that inhalation of *M. edulis* larvae by conspecifics will often lead to the death of the larvae. The effect of inhalation of plantigrade *P. canaliculus* by adults does indeed cause some larval mortality (S. Buchanan, unpubl. data). There is growing acknowledgment that typical mussel recruitment patterns can be more a consequence of primary-settler mortality and postlarval dispersal than an active avoidance by settling larvae. Increasing evidence of primary settlement directly into the adult bed reinforces the notion that the settlement of larvae is highly variable and does not conform to any one particular pattern (Petersen 1984a, Petersen 1984b, McGrath et al. 1988, King et al. 1990, Cáceres-Martínez et al. 1994, Lasiak and Bernard 1995). Larvae may, however, be attracted to the adult bed, despite the inherent risks that this may entail: the attraction of byssus threads and adult shells as sites of settlement has been demonstrated (Eyster and Pechenick 1987, Cáceres-Martínez et al. 1994).

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ABSORPTION EFFICIENCY AND CONDITION OF CULTURED MUSSELS (*MYTILUS EDULIS GALLOPROVINCIALIS* LINNAEUS) OF GALICIA (NW SPAIN) INFECTED BY PARASITES *MARTEILIA REFRINGENS* GRIZEL ET AL. AND *MYTILICOLA INTESTINALIS* STEUER

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ABSTRACT An experiment was performed with cultured mussels (*Mytilus edulis galloprovincialis*) in the Ria de Arousa, NW Spain, under environmental conditions of temperature, salinity, and food availability, in order to determine the effects of *Marteilia refringens* and *Mytilicola intestinalis* on the absorption rate, absorption efficiency, and condition of the mussel. *M. refringens* significantly reduced absorption of organic matter only when the infection was spread throughout the digestive diverticula of the mussel (heavy infection). Moreover, heavy infections by *M. refringens* caused a significant loss of the condition of the mussels, probably as a consequence of reduced energy acquisition. The occurrence of *M. intestinalis* was not associated with reduction of either absorption efficiency or ingestion rate, but infected mussels showed a significantly worse condition.

KEY WORDS: Mussel, *Mytilus*, energetics, absorption, parasites, *Marteilia*, *Mytilicola*

INTRODUCTION

Parameters controlling energy acquisition (rates of feeding and absorption efficiency) in bivalve molluscs show a high variability in response to many factors, both exogenous and endogenous (Bayne and Newell 1983, Bayne et al. 1987, Navarro et al. 1991, Bayne et al. 1993). Sublethal effects of parasitic infections are among those factors (Newell and Barber 1988). Interference by parasitism with physiological mechanisms controlling the energy budget of bivalves has mainly been studied in the Eastern oyster, *Crassostrea virginica* (Gmelin), parasitized by the endoparasites *Haplosporidium nelsoni* (Haskin, Stauber & Mackin) (Newell 1985, Barber et al. 1988a, Barber et al. 1988b, Littlewood and Ford 1990, Barber et al. 1991) and *Perkinsus marinus* (Mackin, Owen & Collier) (Choi et al. 1989) and the ectoparasitic gastropod *Boonea impressa* (Say) (Ward and Langdon 1986, White et al. 1988, Gale et al. 1991). In the case of the blue mussel, *Mytilus edulis*, effects of marine vibrios (McHenerly and Birkbeck 1986), the copepod *Mytilicola intestinalis* (Bayne et al. 1978), and various

parasites and pathological conditions (Gilek et al. 1992) have been studied.

The mussel cultured in the Galician Rías (northwestern [NW] Spain) was traditionally referred to as *Mytilus edulis* (Pérez Camacho et al. 1991). Sanjuán et al. (1990) and Crespo et al. (1990) pointed out that this mussel corresponds to the form *Mytilus galloprovincialis*. However, the taxonomic status of this mussel form is still the subject of discussion, because it is considered to be a true species by some authors (KoeHN 1991, Sanjuán et al. 1990) and to be the subspecies *M. edulis galloprovincialis* by others (Gardner 1992, Gosling 1992). Infections by the protistan *Marteilia refringens* and the copepod *M. intestinalis* are the most significant pathological conditions affecting cultured mussels of the Galician Rías, with regard to prevalence and pathogenicity (Paul 1983, Figueras et al. 1991, Villalba et al. 1993b). Both parasites inhabit the digestive system of their host. *M. refringens* multiplies through the digestive epithelia of mussels, and a wide surface of the digestive diverticula epithelium of the host becomes occupied by parasites in heavy infections (Villalba et al. 1993b). The inhibition of both gonad and storage tissue development of mussels as a consequence of infection by *M. refringens* (Villalba et al. 1993a) can be considered as a sign of broad impairment of mussel physiology. Thus, interference by *M. refringens* with the digestive

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physiology of the mussel should be expected. *M. intestinalis* inhabits mainly the intestinal lumen of the host, and its effects on mussel physiology are controversial (Bayne et al. 1978, Theisen 1987, Davey and Gee 1988). According to the review by Morton (1983), absorption and intracellular digestion of most of the food ingested by bivalves occur in the digestive tubules in the digestive diverticula, whereas the function of the intestine was not well known at that time. Subsequently, Hawkins et al. (1986) demonstrated that substantial absorption of nonchlorophyll organics takes place in the intestine. An experiment was performed to determine whether infections by *M. refringens* and *M. intestinalis* influence the absorption efficiency of the host, and consequently, whether they have an effect on mussel condition.

MATERIALS AND METHODS

The experiment was carried out with cultured mussels on a raft located at the inner part of the Ría de Arousa (Galicia, NW Spain) in late July 1991, under natural environmental conditions of temperature, salinity, and food availability. Forty-six mussels of 78.7 ± 0.8 mm (mean \pm standard error) in length were taken from a culture rope and arranged within individual trays on the raft itself, with continuous seawater flow.

Mussels were permitted to acclimatize for 2 h, and feces produced during this period were removed immediately before the start of the experiment. Subsequently, samples of seawater were taken every 20 min over 3 h for analysis of seston availability. Mussels did not produce pseudofeces during the experiment. Feces produced by each mussel were collected after 1.5 and 3 h, to determine organic and inorganic contents. Samples of both seawater and feces were filtered onto preashed (450°C) and weighed in Whatman glass filters type C and rinsed with isotonic ammonium formate. Total dry matter (DW) was established as the weight increment determined after drying the filters to constant weight at 90°C . Organic matter (OM) corresponded to the weight loss after ignition at 450°C in a muffle furnace for 24 h.

Egestion rates (ER = mg of DW/h) were estimated from the total DW content of the feces. Considering that absorption of inorganic matter (IM) through the digestive system is negligible, ER and ingestion rates (IR) of inorganics were assumed to be identical. Thus, IR were calculated from egestion and organic content of the seston (Navarro et al. 1991, Iglesias et al. 1992).

$$\text{IR} = \text{ER} \times \% \text{IM(f)} / \% \text{IM(s)}$$

where % IM(f) is the fecal inorganic matter content and % IM(s) is the inorganic matter content of the seston. Absorption rates (A) were estimated as the difference between organic ingestion rates (OIR) and organic egestion rates (OER). Absorption efficiencies (AE) corresponded to the ratio A/OIR.

After the completion of physiological determinations, soft tissues of each mussel were excised and a cross-section of tissue was removed and processed for histology (Villalba et al. 1993b). The remaining tissue was weighed wet and then dried (100°C) so that a dry weight/wet weight ratio could be obtained and used to calculate the total dry weight (TDW) of soft tissues of the entire animal (Barber et al. 1988a). The weight of valves was also calculated (SW). A condition index (CI) was calculated as follows (Davenport and Chen 1987):

$$\text{CI} = 100 (\text{TDW}/\text{SW})$$

A histological section of each mussel was examined under light

microscopy to assess the occurrence of *M. intestinalis* and to quantify the intensity of infection by *M. refringens* using the scale of Villalba et al. (1993b). The mussels were distributed within the following classes: noninfected mussels (NI), when neither of the parasites was detected; *Mytilicola*-infected mussels (Myl) when *M. intestinalis* was detected; mussels with light infection (LI) by *M. refringens*, when cells of this parasite were confined to the stomach epithelium or even reached primary ducts; heavily infected mussels (HI) by *M. refringens*, when this parasite was spread through the digestive diverticula and mussels with a mixed infection by both parasites (MIX). No case of moderate infection by *M. refringens* was found among the mussels used in the experiment.

Differences in the percentage of organic content of feces, ER, and absorption among mussels with different intensities of infection by *M. refringens* and *M. intestinalis* were analyzed by means of analysis of variance (ANOVA) of nested samples (Lison 1968, Snedecor and Cochran 1971). Percent data describing organic content were normalized by arcsin transformation. Homogeneity of variances was checked by Bartlett's test. Regressions of $\ln \text{OM}$ Versus $\ln \text{IR}$ for the different infection groups were compared by analysis of covariance (ANCOVA). Differences among CI were analyzed by comparing regressions of DW on SW using ANCOVA (Lison 1968, Snedecor and Cochran 1971). All statistical procedures were performed with STATGRAPHICS software.

In order to use the highest possible number of individuals from the groups with a lower representation, a comparison was made among the fecal organic matter percentage, ER, and IR of the groups NI and Myl; ANOVA did not show significant differences between both groups ($p > 0.05$). Thus, mussels with mixed infection of *M. refringens* and *M. intestinalis* were added to the group of *M. refringens*-infected mussels. Regarding CI, significant differences among NI and Myl were found; therefore, the data for mussels with mixed infection were discarded.

RESULTS

Infection Levels

The distribution of mussels between classes of intensity of infection was as follows: 22 NI, 6 LI, 5 HI, and 9 Myl mussels. The remaining four mussels showed mixed infections.

Characteristics of Seston

Seston characteristics were 0.56 ± 0.08 mg L^{-1} of particulate matter, 0.38 ± 0.05 mg L^{-1} of particulate OM, and an average of 67.5% OM ($n = 8$).

Organic Content of Feces

HI mussels had the highest fecal organic percentage, being very similar in all of the groups (Table 1). ANOVA of nested samples showed significant differences in fecal OM% between HI mussels and all of the other groups ($p < 0.01$). Nonsignificant differences were found between NI-Myl, NI-LI, and Myl-LI.

Ingestion

IR calculated for every infection intensity class are shown in Table 1. Infection with *Marteilia* showed a significant effect on IR. Infected mussels (LI and HI) ingested significantly less food than noninfected (NI and Myl) ($p < 0.05$).

In NI mussels, the percentage of organic matter of the feces

TABLE 1.

Percentage of OMF, IR (mg of DW/h), AE, and absorption rate (A = mg OM/h) of each infection intensity class.

Parameter	NI	LI	HI	MyI
OMF	49.5 ± 1.2	48.6 ± 1.3	57.7 ± 3.0	47.8 ± 1.5
IR	1.66 ± 0.20	1.25 ± 0.12	1.04 ± 0.14	1.62 ± 0.31
AE	0.51 ± 0.03	0.54 ± 0.03	0.31 ± 0.07	0.55 ± 0.04
A	0.65 ± 0.09	0.48 ± 0.06	0.22 ± 0.06	0.68 ± 0.15

Presented values correspond to the means of records at 1.5 and 3 h of sampling ± standard error. See text for explanation of abbreviations.

(OMF) was related to IR (mg of DW/h), according to the double-logarithmic regression model (means ± standard error):

$$\ln \text{OMF} = 3.919 \pm 0.017 - 0.143 \pm 0.021 \ln \text{IR} \\ (r = -0.73, n = 44, p < 0.001)$$

The same model was fitted for the remaining infection classes independently, and the resulting equations were compared by ANCOVA. Significant differences with the MyI and LI infection classes were not found ($p > 0.05$). In contrast, the OMF was independent of IR for the HI mussels ($r = -0.04, p > 0.05$).

Absorption

HI mussels showed a notable decrease in the absorption rate (A; mg/h OM), but the rate was similar between the remaining infection-intensity classes (Table 1). Absorption rate data, compared by ANOVA after logarithmical transformation in order to homogenize HI variances, showed significant differences between the groups HI-NI ($p < 0.05$; 1.54 *df*) and HI-MyI ($p < 0.01$; 1.28 *df*). No significant differences were found between NI-MyI, NI-LI, and LI-MyI ($p > 0.05$).

The AE of HI mussels was considerably lower than those of the other groups (Table 1). The AE of NI, MyI, and LI mussels increased as the ingested food content increased, according to $y = a \cdot e^{(-b/x)}$, whereas it was independent of the IR in HI mussels (Fig. 1).

CI

NI and LI mussels showed the highest CI. The lowest one corresponded to the HI class, whereas MyI mussels showed an intermediate value (Table 2). When regressions of TDW on SW were compared by ANCOVA, significant differences in intercept were found between the groups HI and all other groups and also between NI and MyI (Table 2).

DISCUSSION

Mean values of absorption rates and AE indicate that heavy infections by *M. refringens* significantly reduce the absorption of OM in mussels. Light infections by *M. refringens* did not seem to have marked effects on food absorption, with similar A and AE values for noninfected and lightly infected mussels.

In noninfected mussels, AE increased with IR and became asymptomatic at ingestion values of 2–6 mg/h of DW (Fig. 1), reaching a maximum of about 0.65. A very similar relation was observed for MyI and LI mussels, whereas AE was significantly reduced and independent of food ingestion in HI mussels. This pattern does not agree with studies that showed an inverse rela-

tionship between AE and IR (e.g., Thompson and Bayne 1972, Navarro and Winter 1982). Nevertheless, it has been pointed out that negative correlations derive from laboratory studies carried out with pure phytoplankton, but not from the heterogeneous suspensions occurring in the natural environment (Bayne and Newell 1983). Griffiths (1980) showed that the AE of black mussels did not decrease for seston charges up to 20 mg/L, when feeding on natural detritus. Their result agrees with those of this study.

It has been established that AE is directly related to food organic content, and that for seston concentrations lower than the pseudofeces threshold, AE rises with increasing food quality (Bayne et al. 1987, Navarro et al. 1991). At high particle concentrations (above the pseudofeces threshold), AE can increase with filtration rate as the result of an enrichment of the ingested ration as a consequence of a preingestive selection of organically rich particles (Navarro et al. 1992, Iglesias et al. 1992). However, production of pseudofeces was not observed in this study, and the seston characteristics (both quantity and quality) were the same for all of the mussels, regardless of the quantity of food ingested. Alternatively, the decrease in AE found at decreasing IR (Fig. 1) may be explained as a consequence of the metabolic fecal loss, defined as endogenous material lost from secretion and/or abrasion in the gut (Hawkins et al. 1990). The minimum value of the metabolic fecal loss is the absorption value corresponding to ingestion = 0, i.e., the intercept in the equation relating absorption rate (A) to IR in noninfected mussels: $A = -0.126 \pm 0.021 + 0.471 \pm 0.010 \text{IR}$ ($r = 0.991, n = 44, p < 0.001$). We can now recalculate the actual efficiency of food absorption, disregarding losses of endogenous material, by subtracting this value, 0.126, from the organic content of the feces. This would transform the asymptotic curve of Figure 1 into a straight line parallel to the abscissa axis, $Y = 0.699$, which approximately corresponds to the asymptote of Figure 1. This value is similar to the maximum AE found for mussels from the Ria de Arousa by Navarro et al. (1991).

Intracellular digestion and absorption occur in digestive diverticula (Bayne et al. 1976, Morton 1983). Observation of histological sections of HI mussels under light microscopy has shown that wide areas of the digestive diverticula epithelium are occupied by parasites, whereas the surface of digestive tubules is not increased (Villalba et al. 1993b). Therefore, even assuming that the functionality of the remaining cells in digestive tubules is intact, the functional surface for intracellular digestion and absorption is significantly reduced. In addition, the absorption of food material from tubule lumen or neighbor digestive cells by parasites is likely to occur. That would explain why AE is significantly reduced in HI mussels, but not in LI mussels, in which parasites are confined to stomach epithelium or reach primary ducts at most.

Because energy acquisition is reduced in heavily infected mussels, significant disturbance of mussel physiology can be expected. This could explain the inhibition of both development of storage tissue and gametogenesis in mussels heavily infected by this parasite (Villalba et al. 1993a).

Our results show that mean IR was significantly depressed in mussels heavily and lightly infected by *M. refringens*. Newell (1985) detected significantly lower clearance rates in oysters, *C. virginica*, infected by *H. nelsoni* than in noninfected controls. That author suggested that because *H. nelsoni* multiplies through gill and labial palps, causing a sloughing of their epithelia, disturbance of ciliary function was the most likely cause of clearance rate reduction. However, Barber et al. (1991) did not detect significant differences in clearance rates between oysters infected and non-

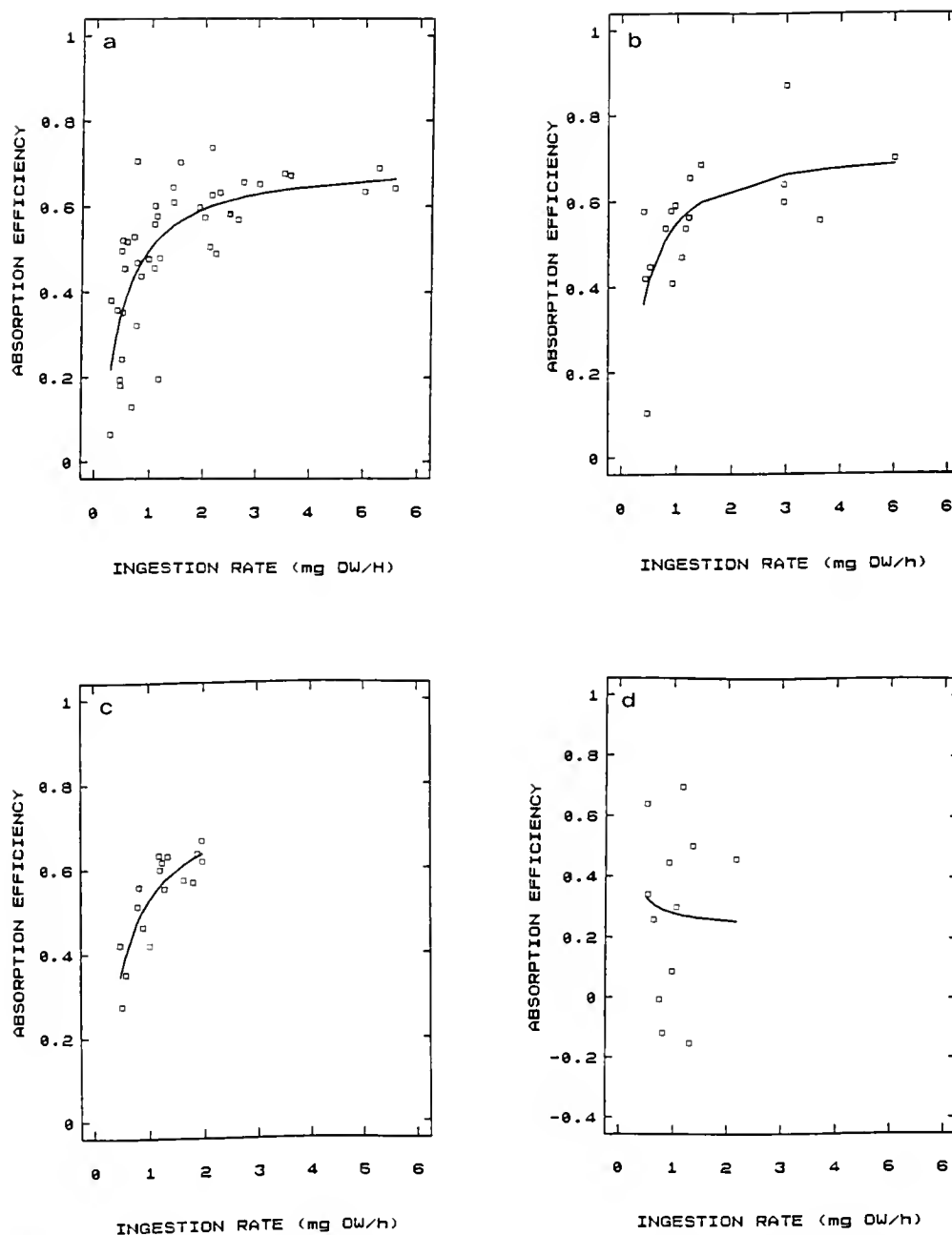


Figure 1. Relationship between AE and IR (mg/h DW) in: (a) noninfected mussels ($AE = 0.7067 \pm 0.039e^{(-0.326 \pm 0.058/IR)}$, $r = 0.73$, $n = 44$); (b) *M. intestinalis*-infected mussels ($AE = 0.731 \pm 0.066e^{(-0.285 \pm 0.090/IR)}$, $r = 0.65$, $n = 18$); (c) mussels lightly infected by *M. refringens* ($AE = 0.774 \pm 0.460e^{(-0.385 \pm 0.650/IR)}$, $r = 0.86$, $n = 18$); (d) mussels heavily infected by *M. refringens* ($AE = 0.991 \pm 0.496e^{(0.875 \pm .875/IR)}$, $r = 0.07$, $n = 12$).

fectured by *H. nelsoni*. McHenry and Birkbeck (1986) also reported inhibition of filtration in *M. edulis* by marine vibrios, suggesting that some bacterial ciliostatic toxin could be the cause. *M. refringens* does not proliferate through either gill or labial palp tissues; therefore, a physical interference with the normal function of the filtration system by *M. refringens* should not be expected. Nevertheless, the reduction of IR detected in this experiment concomitantly with infection could be considered as a compensatory response induced by the limited functional capability of the digestive system (Bayne et al. 1987, Navarro et al. 1994).

The significant decline in CI in HI mussels could also be explained by the reduction of CI in the rate of energy acquisition.

Loss of condition caused by protistan parasites has also been described in oysters, *C. virginica*, (Newell 1985, Barber et al. 1988a, Choi et al. 1989) and *Ostrea edulis* (Robert et al. 1991). Similar effects are caused by metazoan parasites in bivalves (reviewed by Lauckner 1983).

The effects of *M. intestinalis* on mussels are controversial. This copepod was blamed for mass mortalities in European mussel beds before 1970 and, consequently, was considered as a pest for mussel populations. However, more recent studies, based on a more detailed knowledge of mussel physiology, have led others to attribute minimum detrimental effects to this parasite (reviewed by Davey and Gee 1988). Gee et al. (1977) found some effect of *M.*

TABLE 2.

DW-SW linear regression parameters, CI \pm SE, and ANCOVA between DW (dependent variable) and SW (independent variable) of mussels with different types of infection.

	Regressinn DW-SW				ANCOVA					
	NI	LI	III	MyI	III-NI	III-MyI	III-LI	MyI-NI	MyI-LI	LI-NI
Intercept	-0.033	-0.151	-0.307	3.540						
Slope	0.264	0.261	0.161	-0.123						
CI	26.0 \pm 0.99	24.7 \pm 3.60	13.0 \pm 2.33	21.6 \pm 1.62						
Intercept										
F					29.752	8.484	6.751	5.971		
df					1,24	1,11	1,8	1,28		
S.L.					0.000	0.014	0.032	0.021	NS	NS
Slope										
S.L.					NS	NS	NS	NS	NS	NS

SW, shell weight; S.L., significance level; df, degrees of freedom, $F = F$ ratio; NS, S.L. > 0.05.

intestinalis on the CI of mussels, but only in winter months, when the mean number of parasites per host was over 25. Bayne et al. (1978) reported inhibition of feeding rate by this parasite only when *M. edulis* had more than 10 parasites, under conditions of high temperature and low ration. Results from our experiment indicated that infection by *M. intestinalis* did not reduce either IR or AE, but CI was significantly reduced in infected mussels at the experimental conditions, probably because of competition for food energy. Theisen (1987) concluded that *M. intestinalis* has a strong adverse effect on the condition of its host, *M. edulis*. That author stated that this effect is masked in individual samples with large variation in the condition of the host by the fact that mussels with higher CI are able to lodge more copepods than mussels in poorer condition. In the case of mussels cultured in Galician Rías, Paul (1983) found significant effects on host CI by the occurrence of the copepod in only a few samples, mainly in spring and summer, after the main spawning period. That author suggested that heavy burdens of copepods may affect the ability of mussels to recover from

spawning. The prevalence of this parasite estimated from our mussels was much lower than that 80–100% found by Paul (1983) in cultured mussels from the same location. The usual method for detection of this parasite involves the dissection of the whole mussel. We examined a 6- μ m-thick histological section instead, and thus, the occurrence of *M. intestinalis* was probably only detected in mussels with a high burden of parasites. The method used for parasite detection and the fact that our experiment was accomplished in late July, when the mussel reproductive season ends (Villalba et al. 1993a), could explain the detection of a significant effect on mussel condition by *M. intestinalis*.

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MUSSEL (*MYTILUS GALLOPROVINCIALIS* LAMARCK) SETTLEMENT IN THE RIA DE VIGO (NW SPAIN) DURING A TIDAL CYCLE

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ABSTRACT The settlement of mussel was determined during a tidal cycle in an exposed rocky shore in the Ria de Vigo (north-western Spain) and 300 m away from it. In the exposed rocky shore, mussel settlement was recorded throughout the intertidal profile during the tidal cycle. Settlement was more abundant in the lower than in the upper intertidal zone. The size of settled mussels varied from 0.250 to 11 mm. The largest mussels were found in the lower intertidal zone. Maximum densities were recorded during the high tide, and the minimum were recorded during the low tide. Three hundred meters away from the mussel bed, settlement occurred during the complete tidal cycle. A light increase in the number of settled mussels during high tide was recorded. Settlement was more abundant at 2- than at 5- and at 8-m depth. The size of settled mussels varied from 0.225 to 0.375 mm and was similar at all depths studied.

KEY WORDS: *Mytilus galloprovincialis*, settlement, tidal cycle

INTRODUCTION

Mussel settlement has been widely studied under very different conditions (Maas-Geesteranus 1942, de Blok and Geelen 1958, Böhle 1971, Dare 1973, Dare 1976, Hrs-Brenko 1973, Dare et al. 1983, Sigurdsson et al. 1976, Petersen 1984, King et al. 1989, Newell et al. 1991, Cáceres-Martínez et al. 1993, Cáceres-Martínez et al. 1994, McGrath et al. 1994). Mussel larvae distribution during tidal cycles have been studied by Newell et al. (1991). However, to our knowledge, no studies on mussel settlement during a tidal cycle have been done. The aim of this work was to determine variation in the number of settling mussels (*Mytilus galloprovincialis*) during a tidal cycle through the intertidal zone in a mussel bed and in a sampling location 300 m away from it.

MATERIALS AND METHODS

In the summer of 1993, when major settlement of mussels occurs in the area (Cáceres-Martínez et al. 1993, Cáceres-Martínez et al. 1994), sampling of the mussel settlement during a tidal cycle, from July 21 to 22, in the exposed rocky shore of Cabo Home, on the oceanic side of the Ria de Vigo (42°5'N, 8°52'W), was carried out. Pieces of 19 × 16 × 0.8 cm of synthetic fibrous material (Commercial Scotch Brite™) were used as collectors. A pulley system was placed in the exposed rocky shore, from the low-water spring tide level mark to the high-water spring tide level mark. A series of six collectors by duplicate were hung from the polyethylene ropes (0.5 cm in diameter) every 5 m; additional weight for collectors was not required. These collectors were replaced three times: 6 h after the first low tide, 6 h after the high tide, and 6 h after the following low tide. Simultaneously, a series of three collectors were submerged at 2-, 5-, and 8-m depth from a boat anchored at 300 m in front of the rocky shore (sublittoral site), and these were replaced every 2 h. Two replicates were made for each collector. The tidal heights in meters were estimated from the lower water tidal height mark recorded for June 21, 1993, in the

tide tables (Junta del Puerto y Ria de Vigo 1993). The low-water tide level is defined as zero in tidal data.

To separate spat, each collector was immersed for 5 min in a 10% solution of commercial sodium hypochlorite (Na ClO) and rinsed in tap water onto a series of 0.09- to 4.0-mm sieves. The resulting fractions were dried in an oven at 80°C for 24 h. Mussels were separated with a brush for study under a stereoscopic microscope, and all mussels in a fraction were counted. The mussels obtained in the sieves under 3-mm mesh were measured with a micrometer (total shell length). Larger mussels were measured with a caliper to the nearest 0.5 mm. Results are presented as the number of individuals per collector.

STATISTICAL ANALYSIS

A Kruskal-Wallis test followed by Tukey-type multiple comparisons (Zar 1984) was used for comparisons in the settlement in different localities. A *t*-test and one-way analysis of variance (ANOVA) to Wilcoxon signed rank test were used to compare size composition in different localities (Sokal and Rohlf 1981).

RESULTS AND DISCUSSION

Mussel Settlement in the Rocky Intertidal Zone Over a Tidal Cycle

Mussel settlement occurred at each sampling site in the intertidal zone. The abundance was higher after the flood of the tide than after the ebb, suggesting that tide and waves may carry mussels to the shore and transport them again to the open sea (Fig. 1). This is similar to barnacle dispersion during their settlement process (de Wolf 1973). Cyprids are transported by tidal currents, sinking at that time to the bottom during periods of low current speed and then being dispersed again in the water column when water speed increases. Newell et al. (1991) found that mussel larvae are more abundant on the flood tides, indicating inshore and estuarine retention. In this study, the minimum and maximum mussel sizes recorded were, respectively, 0.225 and 11.0 mm. Interestingly, mussels larger than 10.0 mm were found attached to collectors. Their presence may be explained by the capacity of bivalve and gastropod postlarvae stages up to 2 mm to produce the contact mucous threads, also named byssus threads (Sigurdsson et al. 1976, de Blok and Tan Mass 1977), that are used to make contact with the substrate, allowing settlement and providing ad-

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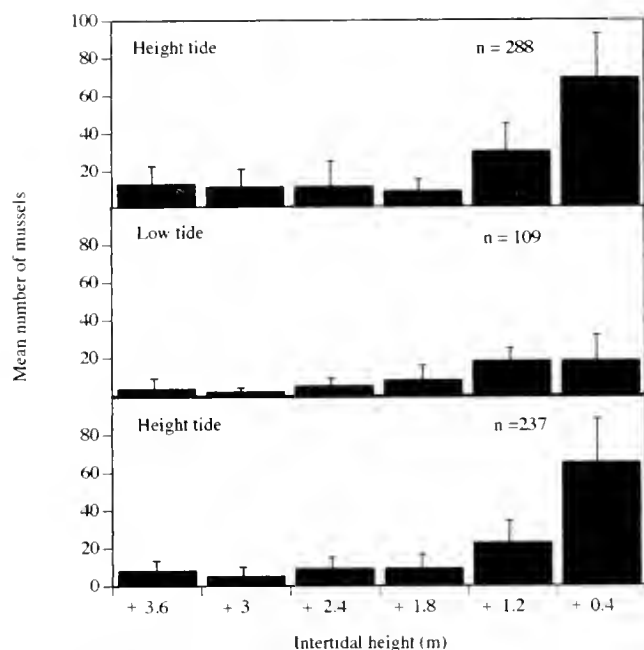


Figure 1. Mean number of mussels settled (+standard deviation) on collectors placed at different heights of the intertidal profile of an exposed rocky shore during a tide cycle in Ria de Vigo, Spain.

ditional buoyancy for dispersion. (Beukema and de Vlas 1989, Martel and Chia 1991, Cáceres-Martínez et al. 1994). In mussels, the superior limit in the size of individuals with the capacity to produce these mucous threads has not been established; our results suggest that this size is around 10 mm. Similarly, Beukema and de Vlas (1989) found *Macoma balthica* of a shell length of 10 mm with these mucous threads. The range of mussel sizes found on the lower intertidal zone was wider than that on the upper zone (Fig. 2). The number of mussels settled on collectors increased from the upper to the lower intertidal zone. This was corroborated by a regression between tidal height and total number of settled mussels, which was significant ($y = 95.047 - 8.9x$, $R^2 = 0.7$, $p < 0.01$). If settlement depends on a chance encounter between the mussel and the appropriate substrate, one reason for higher mussel abundance in collectors placed at the lower intertidal zone than at the upper one could be the longer immersion time period of these collectors. On the other hand, the buoyancy of pediveliger stages, marked by the coexistence of velum, foot (Widdows 1991), and contact mucous thread (Sigurdsson et al. 1976, de Blok and Tan Mass 1977, Cáceres-Martínez et al. 1994), seems to be greater than the buoyancy of postlarvae and larger mussels (>0.470–11 mm) (Bayne 1971). This could explain why postlarvae and larger mussels are more abundant in the lower intertidal zone than in the upper one.

Spat Abundance at Different Depths During the Tidal Cycle

Spat abundance recorded during the tidal cycle showed an irregular pattern at the sublittoral site. However, an increase in spat after high tide was detected, especially in the collector placed at 2 m (Fig. 3). Spat were more abundant at 2- than at 8-m depth, and this was statistically significant (Kruskal-Wallis test, $H = 8.502$, $p < 0.01$, followed by Tukey-type multiple comparisons). This could be explained by two hypotheses: (1) collectors placed at 8-m depth were dragged by the waves on the sandy bottom, limiting

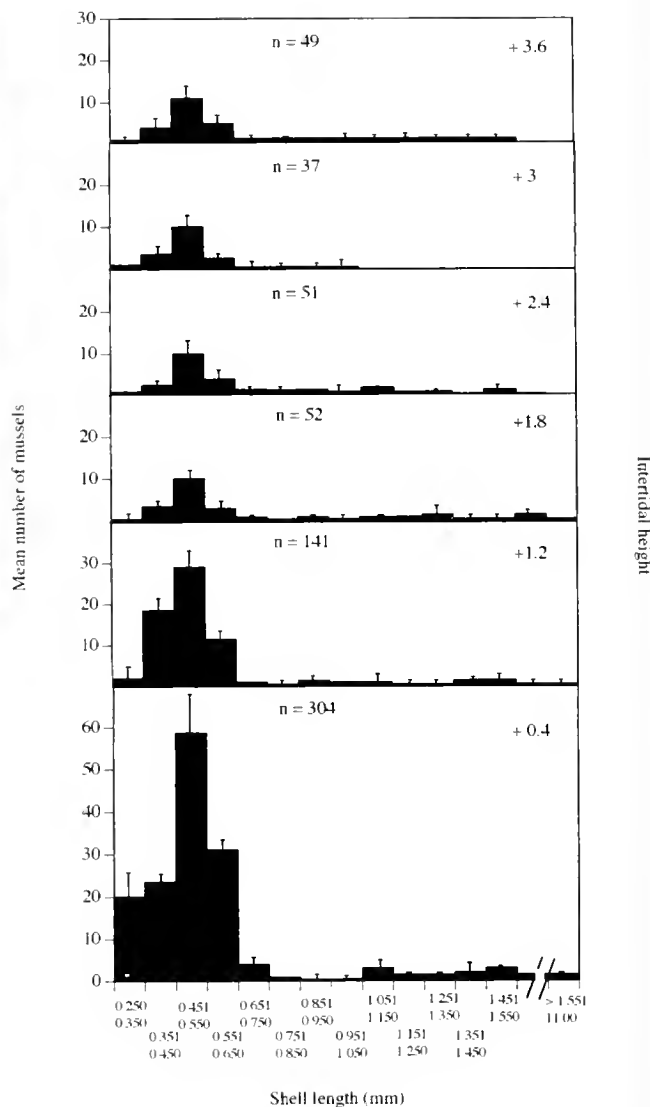


Figure 2. Size distribution of mussels settled on collectors placed at different heights of the intertidal profile of an exposed rocky shore during a tide cycle in Ria de Vigo, Spain. (+standard deviation).

attachment as the result of friction against the substrate and/or (2) ascending (tidal) currents occurred at that moment. It is known that bivalve larvae vertical distribution may respond to tidally induced cues such as changes in pressure, temperature, or salinity. How-

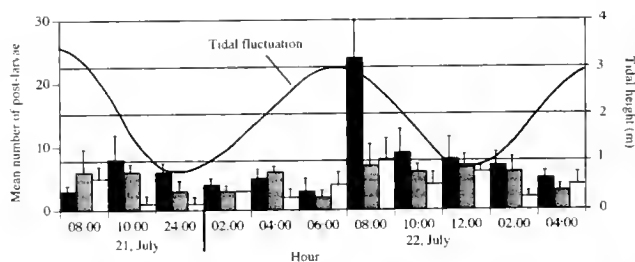


Figure 3. Mean number of mussels settled (+standard deviation) on collectors placed at 2- (black bars), 5- (gray bars) and 8-m (white bars) depth, during a 24-h cycle in Ria de Vigo, Spain. The black line indicates the tidal fluctuation in depth (m).

ever, this responses may be overridden by the energy of the system, and the larvae behave as inanimate particles in their distribution (Newell et al. 1991).

Interestingly, the minimum and maximum sizes recorded on collectors placed at the sublittoral site were 0.225 and 0.375 mm (corresponding to pediveliger larvae stages), respectively. No statistically significant differences were detected among the sizes of mussels settling at different depths (one-way ANOVA, $F = 0.064$, $p > 0.05$). However, there were statistically significant differences between the sizes of the mussel spat from the sublittoral site (0.225 and 0.375 mm) and those placed in the intertidal zone (0.225–11.0 mm) (t -test, $p < 0.001$). The phenomenon of mussel postlarvae dispersion is not entirely understood. Several authors (Böhle 1971, Hrs-Brenko 1973, Rees 1954, Kautsky 1982) found very few mussel larvae larger than 300 μm in plankton hauls in several studies carried out in very different areas, concluding that postlarvae were absent or scarcely present in plankton and disregarding the occurrence in this species of planktonic postlarvae dispersion. Our results suggest that postlarvae dispersion occurs mainly at a local level, especially in the lower intertidal zone (see above), where the

mussel bed is dense and postlarvae attached to suboptimal substrates may be continuously detached (Cáceres-Martínez et al. 1994). The early life strategy of the mussel, with its planktotrophic existence, accounts for the high dispersal capability of most species within the genus *Mytilus* (Lutz and Kenish 1992). Additional dispersion of postlarvae stages provides the species with a more or less local redistribution possibility. Further research on the factors that control the postlarvae dispersion process and its ecological significance, among them, settlement studies during tidal cycles, is needed.

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FLUORESCENCE IN SITU HYBRIDIZATION OF VERTEBRATE TELOMERE SEQUENCE TO CHROMOSOME ENDS OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS* THUNBERG

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ABSTRACT Fluorescence in situ hybridization (FISH) is useful in genomic research. We tested FISH in the Pacific oyster, *Crassostrea gigas* Thunberg, using metaphase chromosomes prepared from early embryos and all-human telomere and centromere probes. FISH with the all-human telomere probe produced strong hybridization signals at ends of all oyster chromosomes, suggesting that: (1) chromosomes from embryo preparation are suitable for FISH analysis; and (2) the vertebrate telomere sequence, $(T_2AG_3)_n$, may be present in telomeres of the Pacific oyster. No interstitial sites were detected for the telomere sequence. FISH with the all-human centromere probe failed to detect any complementary sequences in oyster chromosomes.

KEY WORDS: FISH, chromosome, telomere sequence, gene mapping, mollusc, *Crassostrea gigas*

INTRODUCTION

Fluorescence in situ hybridization (FISH) is a powerful tool in genomic analysis. By visualizing hybridization sites of a specific DNA probe, FISH permits the direct mapping of genes or DNA fragments to specific chromosomes and subchromosomal regions. Today, FISH is used in a variety of applications including the characterization and identification of chromosomes, the detection of aneuploidy, the physical mapping of genes and DNA fragments, the determination of linkage order, the detection of chromosomal deletions and arrangements, and comparative genome hybridization (Kallioniemi et al. 1992, Chowdhary et al. 1995, Matsuda and Chapman 1995, Wang et al. 1995, Pedersen et al. 1996).

Despite the active use of FISH in other taxa, there have been few studies on FISH in molluscs. Only one study of FISH has been reported in oysters, where a 166-base-pair (bp) of satellite repeats was localized to two chromosomes (Clabby et al. 1996). FISH in molluscs is generally limited by a lack of reliable protocols and probes, not by a lack of interest. In fact, several important areas of genomic manipulation and analysis in molluscs have been difficult in the absence of FISH technology. One example is aneuploid research in oysters. Many types of aneuploids are viable and can be reliably produced in oysters (Guo et al. 1992a, Guo et al. 1992b, Guo and Allen 1994a). Some aneuploids, such as monosomics and trisomics, are especially useful for the identification and chromosomal assignment of quantitative trait loci. The major obstacle in the development and use of aneuploid lines has been the inability to identify specific aneuploids, because chromosome identification by traditional banding is time consuming and less reliable in oysters than in other taxa. The development of FISH protocols and probes may provide effective methods of chromosome identification and pave ways for aneuploid research in oysters.

One of the challenges for FISH and other chromosomal analyses in oysters is the difficulty to consistently obtain metaphase chromosomes. Because cell or tissue culture is not yet possible in oysters, chromosomes have to be prepared from adult tissues or embryos. Adult tissues usually have a low mitotic index and produce highly contracted chromosomes. Although metaphase chromosomes can be more reliably obtained from early embryos, one major concern is whether the yolk materials from early embryos, which were inhibitory to trypsin G-banding, would also inhibit FISH. In this study, we tested FISH on Pacific oyster chromo-

somes obtained from early embryos, using all-human telomere and centromere probes.

MATERIALS AND METHODS

Oyster metaphase chromosomes were obtained from 4-h-old embryos cultured at 25°C (Guo et al. 1992a). Eggs and sperm were obtained from mature oyster by stripping gonads. Eggs were passed through a 60- μ m nylon screen to remove large tissue debris and rinsed on a 20- μ m screen. Eggs were resuspended in seawater and fertilized by adding sperm suspension. Excessive sperm were removed at 15 min postfertilization (PF) by rinsing fertilized eggs on a 20- μ m screen. Embryos were resuspended and cultured for 4 h at 25°C. At about 4 h PF, embryos were harvested and treated with 0.005% colchicine for 15 min. After the removal of colchicine, nine parts of 0.075 M KCl were added to every part of embryo suspension in a hypotonic treatment lasting for 8–10 min. Embryos were fixed with 1:3 acetic acid and methanol and stored at 4°C. For slide preparation, three drops of embryo suspension were loaded on each slide and air-dried at 45° angle. When more spreading is desired, slides were flooded with three drops of 1:1 methanol and acetic acid before drying. Slides were aged for 7 days before FISH analysis.

FISH was conducted according to a protocol recommended by Oncor, Inc. (Gaithersburg, MD). Slides were pretreated with 2 \times SSC (pH 7.0) for 30 min at 37°C, dehydrated successively in 70, 80, and 95% ethanol for 2 min each, and air-dried. Denaturation was done by immersing slides in 70°C denaturation solution for 2 min. The denaturation solution consisted of one part of 20 \times SSC, two parts of distilled water, and seven parts of formamide. Slides were dehydrated in cold ethanol immediately after denaturation.

Two digoxigenin-labeled probes were tested on oyster chromosomes in this study; both were supplied by Oncor, Inc. One was an all-human telomere probe, $(T_2AG_3)_n$, (Cat.# P5097), and the other was an all-human centromere probe (Cat.# P5095). Both probes were labeled with digoxigenin and came in Hybrisol VI (50% formamide, 2 \times SSC). Probes were denatured by incubation at 70°C for 5 min. Denatured probes were placed on ice until use. For hybridization, 30 μ L of probes was placed on each slide, covered with a 22 \times 50 mm glass coverslip, and sealed with rubber cement. Slides were incubated at 37°C for 1–2 h in a humidified chamber. After hybridization, slides were washed in 72°C 2 \times SSC for 5 min

and stored in 1× PBD (phosphate-buffered detergent; Oncor Cat.# S1370-7). Hybridization was detected with a digoxigenin-fluorescein isothiocyanate detection kit (Oncor Cat.# S1331-DF). Sixty microliters of detection reagent was applied to each slide, covered with a plastic coverslip, and incubated at 37°C for 5 min. Detection reagent was washed three times with 1× PBD. Slides were counterstained with 18 mL of propidium iodide/antifade, covered with a coverglass, and readied for viewing. Ektachrome color slide film (400 ASA) was used for documentation.

RESULTS

Hybridization with the all-human telomere probe produced strong signals on termini of all oyster chromosomes (Fig. 1A). Washes at higher stringency (0.5X SSC) did not affect the hybridization of the telomere probe to oyster chromosomes. Hybridization signals located exclusively at chromosome ends, and no interstitial sites were detected. Signals were weak in one or two chromosomal ends, probably as a result of the random variations in hybridization conditions. For some chromosomes, it was noticed that the hybridization signal on one of the sister chromosomes was stronger than the other.

FISH with the all-human centromere probe failed to yield any hybridization sites on oyster chromosomes. To assure that the failure was not due to poor probe quality, we tested the all-human centromere probe on human metaphase chromosomes and obtained strong hybridization signals at the centromeres of human chromosomes (Fig. 1B).

DISCUSSION

For oysters and many other marine molluscs, early embryos represent the best source for metaphase chromosomes (Guo et al. 1992a, Guo and Allen 1994b). Results with the telomere probe in this study clearly demonstrate that metaphases prepared from early embryos are suitable for FISH analysis. The yolk material, which is problematic for trypsin G-banding, did not inhibit DNA hybridization and detection. The weak signals on a few chromosomes may be caused by random variation in hybridization conditions or by variation in the amount of telomere DNA among telomeres.

Telomeres are the terminal protein-DNA structure located at

the ends of all eukaryotic chromosomes. They protect linear chromosomes from DNA degradation, end-to-end fusion, rearrangements, and chromosome loss (Lewin 1994, Zakian 1995). The DNA component of telomeres usually consists of repeats of a simple sequence about 5–10 bp in length. Telomere sequences are highly conserved through evolution. All vertebrates studied so far, as well as the protozoa *Trypanosoma* and several slime molds and fungi, share the same telomere sequence, $(T_2AG_3)_n$ (Zakian 1995). Although telomere sequences in invertebrates are more variable, they share some similarities with each other and the vertebrate sequence (Zakian 1995).

The successful hybridization of the all-human telomere probe to termini of oyster chromosomes strongly suggests that the vertebrate telomere sequence, $(T_2AG_3)_n$, may be present in the Pacific oyster. It is possible that hybridization signals seen in this study were due to cross-hybridization of the vertebrate telomere sequence to a similar, but different oyster telomere sequence. Cross-hybridization is usually eliminated by intensive washing, but washes at higher stringency in this study seemed to have no effect on signal intensity. Therefore, it is likely the vertebrate telomere sequence does exist in the Pacific oysters. Although telomere sequences are unknown in most marine invertebrates, two marine worms (Polychaeta) have been shown by FISH to contain the vertebrate sequence (An et al. 1995). An insect telomere sequence, $(T_2AG_2)_n$, has been identified in the silkworm (*Bombyx mori*) and many other insects (Okazaki et al. 1993). Bulldog ants (*Myrmecia*: Formicidae), however, have both the insect and the vertebrate sequences in their telomere region (Meyne et al. 1995). Cloning and sequencing studies are needed to define the oyster telomere sequence(s), and this study suggests that the human telomere sequence can be used as a probe to screen genomic libraries for oyster telomere DNA.

The failure for the all-human centromere probe to hybridize with oyster chromosomes is understandable. Oncor's all-human centromere probe consists of a selection of chromosome-specific α -satellite sequences—tandem repeats of 171-bp units (Oncor Catalog). Most of these chromosome-specific sequences are designed to prevent cross-hybridization with other human chromosomes or chromosomes from another taxa.

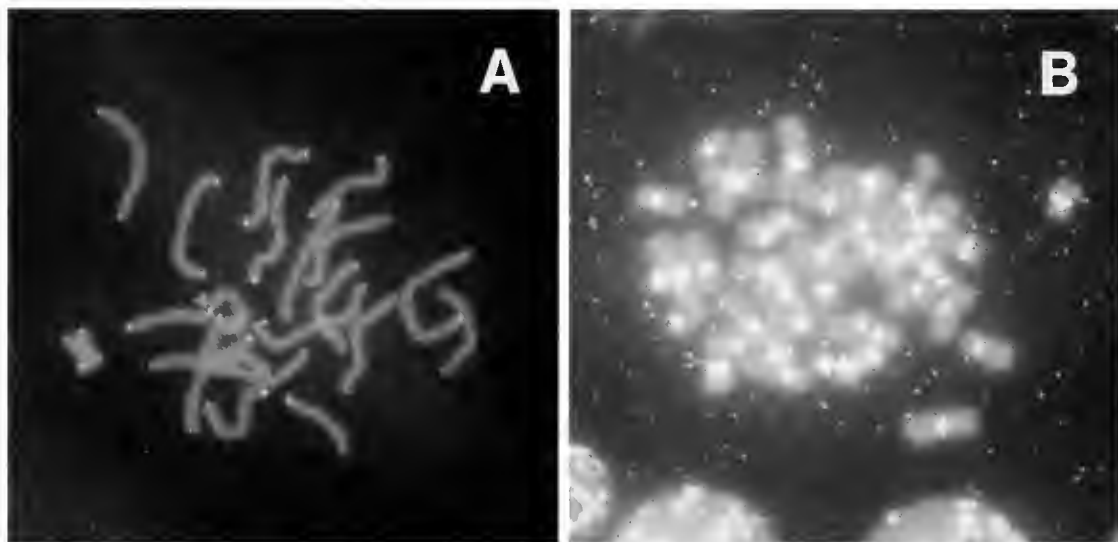


Figure 1. FISH of the all-human telomere probe to ends of oyster chromosomes (A) and the all-human centromere probe to human chromosomes (B).

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ANNUAL PATTERN OF SETTLEMENT IN POPULATIONS OF CHILEAN OYSTERS *TIOSTREA CHILENSIS* (PHILIPPI, 1845) FROM NORTHERN NEW ZEALAND

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ABSTRACT Patterns of larval settlement were examined in two populations of Chilean oysters, *Tiostrea chilensis*, in northern New Zealand. Artificial settlement surfaces were used to measure larval settlement rates over 36 mo at one site and 20 mo at the other. Larvae settled at both sites throughout the year, but with distinct peaks beginning in late winter and early spring and often continuing into early summer. This pattern was unlike those found previously in *T. chilensis* populations at higher latitudes. The annual pattern of larval settlement was closely related to the pattern of oyster brooding over the same period. Overall, the results suggest that colder water temperatures during winter (i.e., below 12°C) are important in synchronizing the annual cycle of larval production and settlement in this oyster species.

KEY WORDS: Chilean oyster, *Tiostrea chilensis*, settlement, larvae, New Zealand, flat oyster, *Ostreidae*

INTRODUCTION

Reproduction in the Chilean oyster *Tiostrea chilensis* (Philippi, 1845) is characterized by an extended incubation period culminating in the release of benthopelagic larvae that are capable of settling immediately (Hollis 1962, 1963, Millar and Hollis 1963, Cranfield 1968b, Bull 1971, Stead 1971, Westerskov 1980, Jeffs and Creese 1996). Most larvae are thought to settle within minutes of release, although in some populations of oysters, a small proportion of the larvae may become planktonic for up to 11 days (Cranfield 1968a, Cranfield 1968b, Stead 1971, Walne 1974, Westerskov 1980, DiSalvo et al. 1983, Cranfield and Michael 1989). Consequently, it could be expected that the annual pattern of larval settlement should closely follow the annual brooding cycle. This has been demonstrated for the short annual period of brooding and settlement in populations of *T. chilensis* in southern New Zealand (Cranfield and Allen 1977, Westerskov 1980).

Previously we described marked differences in the annual pattern of brooding for this oyster from two sites in northern New Zealand, leading us to conclude that water temperature was important in regulating the annual pattern of reproduction in this species (Jeffs et al. 1996). In this article, we report on the annual pattern of larval settlement in these two populations in relation to water temperatures.

MATERIALS AND METHODS

The location of the two study populations of Chilean oysters in the Manukau Harbour and Hauraki Gulf have been described previously (Jeffs et al. 1996, Jeffs et al. 1997). Artificial settlement surfaces were deployed at each site to measure the intensity of oyster spatfall. This technique has been used in previous studies of *T. chilensis* and for other oyster species (Cole and Knight-Jones 1939, Korringa 1941, Shaw 1967, Cranfield 1968a, Cranfield

1968b, Cranfield 1970, Bull 1971, Hickman 1987). The artificial settlement surfaces consisted of cement-fiber board cut into plates that each measured 310 × 295 × 5 mm. Frames were used to hold replicate plates horizontal, with the first plate positioned 40 mm from the seafloor and with subsequent plates spaced at 20 mm intervals above this. Each frame was permanently anchored to the seafloor with buried weight. Replicate frames were 10 m apart and placed within the main oyster bed. Three frames each holding three plates were deployed in the Manukau Harbour, and two frames each with four plates were deployed in the Hauraki Gulf. At monthly intervals, plates were removed from the frames and replaced with clean plates. All oyster spat, both dead and alive, on each plate were identified and counted with the aid of a stereomicroscope. Spat that died soon after settlement on the cement-fiber plates persisted because the lower valves of the prodissoconchs were always well cemented to the settlement plate. Therefore, the counts of settled spat could be expected to provide an accurate measure of the total number of spat arriving on the settlement plates for the period they were exposed. The mean number of spat for the sides of all settlement plates was calculated for each monthly sample, and for comparative purposes, the means were standardized as the number of spat settling per day of plate exposure for a square meter of settlement surface (i.e., #spat day⁻¹ m⁻²). Sampling began in December 1992 in the Manukau Harbour and in April 1994 in the Hauraki Gulf. For both populations, sampling continued until December 1995.

For the duration of the study, water temperatures at each site were measured with handheld thermometers to the nearest 0.1°C when researchers visited the study sites. The results of some of these measurements were reported previously (Jeffs et al. 1996). In addition, remote temperature-recording equipment (Data-sonde™ and Dataflow™) was deployed at both sites for the final

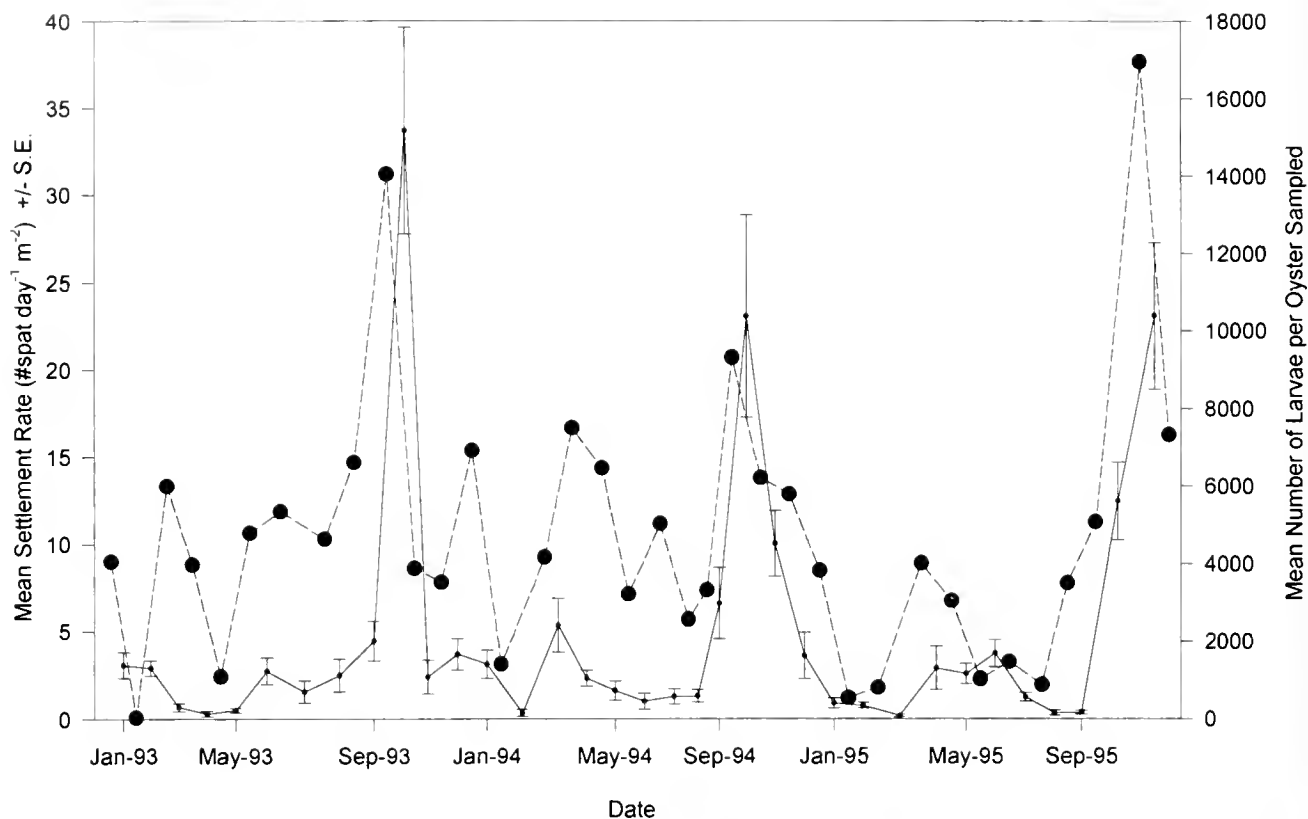


Figure 1. The mean rate of larval settlement for monthly samples in the Manukau Harbour, with corresponding monthly estimates of larval production (from Jeffs et al. 1996a).

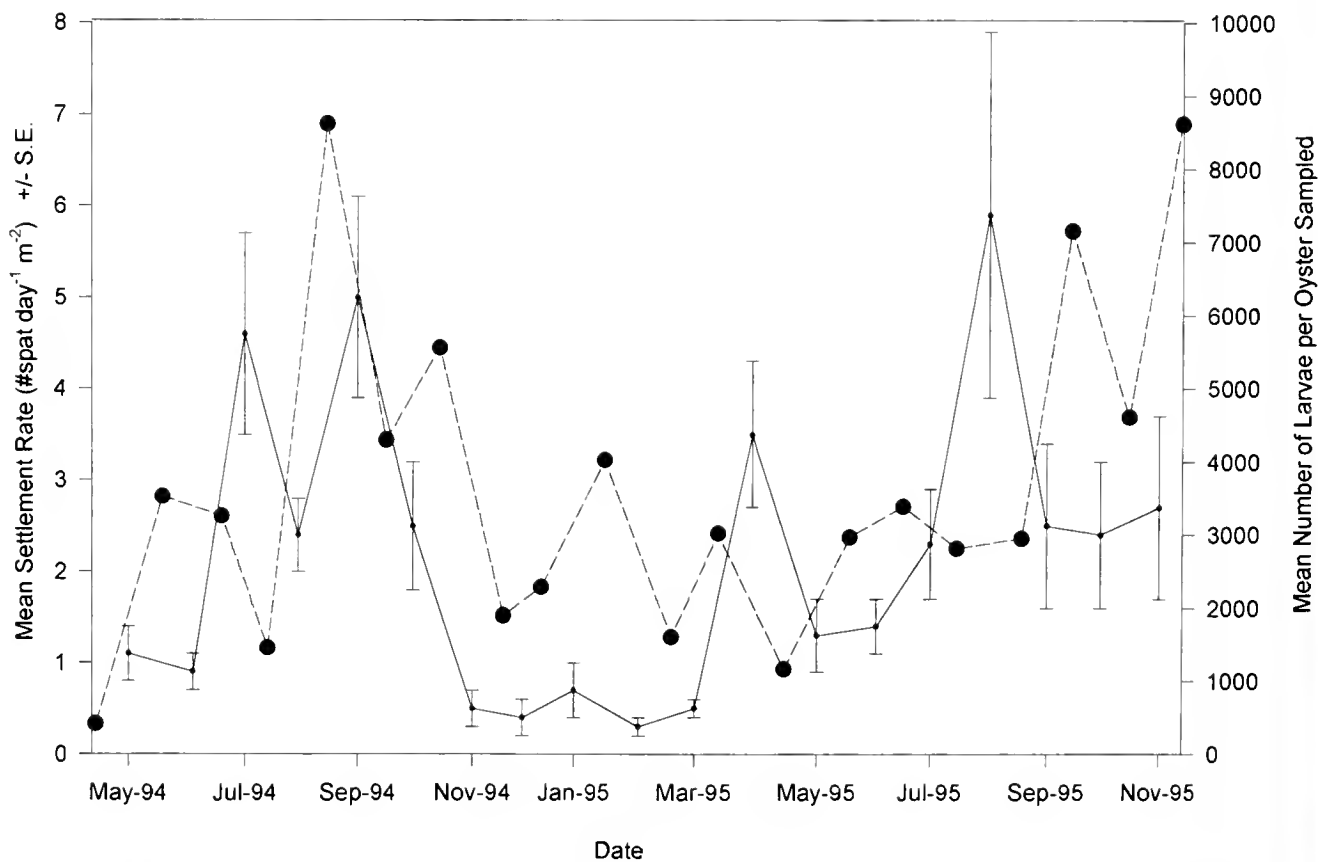


Figure 2. The mean rate of larval settlement for monthly samples in the Hauraki Gulf, with corresponding monthly estimates of larval production (from Jeffs et al. 1996a).

year of the study. These instruments recorded water temperature to the nearest 0.01°C at 30 min intervals. Mean monthly temperatures were calculated for handheld thermometer recordings, and mean weekly temperatures were calculated for remote temperature recordings.

RESULTS

Annual Pattern of Larval Settlement

At both sites, *T. chilensis* larvae were settling in every month of sampling. There were distinct peaks of settlement at both sites, although the peaks were much more pronounced in the Manukau Harbour (Fig. 1). Larval settlement peaked in the Manukau Harbour from September to November of each year (Fig. 1). Peaks of larval settlement were found earlier in the Hauraki Gulf, from July to November of each year, with a further smaller peak in March to April 1995 (Fig. 2).

The annual pattern of larval settlement at our two study sites corresponded closely with the annual pattern of larval production observed in these populations over the same period (Figs. 1 and 2; Jeffs et al. 1996). This relationship was particularly close for the Manukau Harbour population, albeit with a delay of 1 mo. For example, both the amplitude and the timing of the three annual peaks of settlement corresponded with those observed for larval production.

At the Hauraki Gulf population, the relationship between the annual pattern of larval production and larval settlement was not as conspicuous. The peaks of larval settlement were less pronounced than in the Manukau Harbour, as were the peaks of larval production. Also, the peaks of larval settlement at this site appeared to follow peaks of brooding activity by 2 mo rather than 1 mo (Fig. 2).

Annual Pattern of Water Temperatures

At both sites, the mean weekly water temperatures for the year of 1994–1995 fluctuated seasonally, with the highest mean temperatures for both sites in the summer month of February (Figs. 3 and 4). The lowest mean temperatures were in August for the Hauraki Gulf and during July in the Manukau Harbour. The highest and lowest temperatures recorded in this year were also during these months (Figs. 3 and 4). Temperatures over the year of 1994–1995 were much more variable in the Manukau Harbour than in the Hauraki Gulf, as reflected in the greatest recorded temperature changes in 24 h (Figs. 3 and 4). Daily temperature fluctuations in the Manukau Harbour were associated with periods of spring and neap tides and were probably caused by the influx of cooler oceanic waters and insolation of the harbor's shallow waters. Also, the oyster bed in the Manukau Harbour was exposed for short periods to ambient air temperatures during spring low tides. These periods

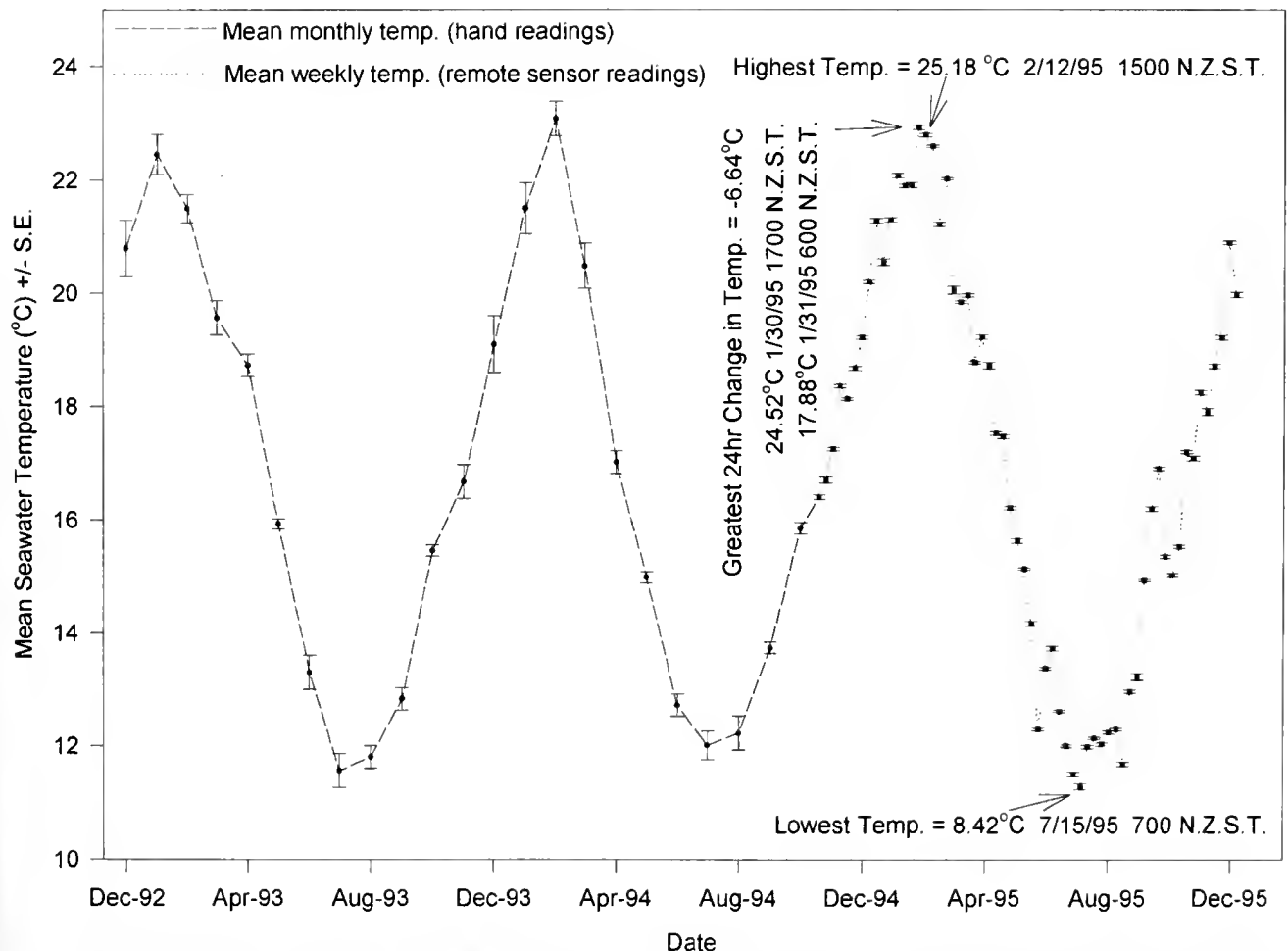


Figure 3. Mean water temperatures (temp.) in the Manukau Harbour from 1992 to 1995. (N.Z.S.T. = New Zealand Standard Time = Greenwich Mean Time +12 hrs.)

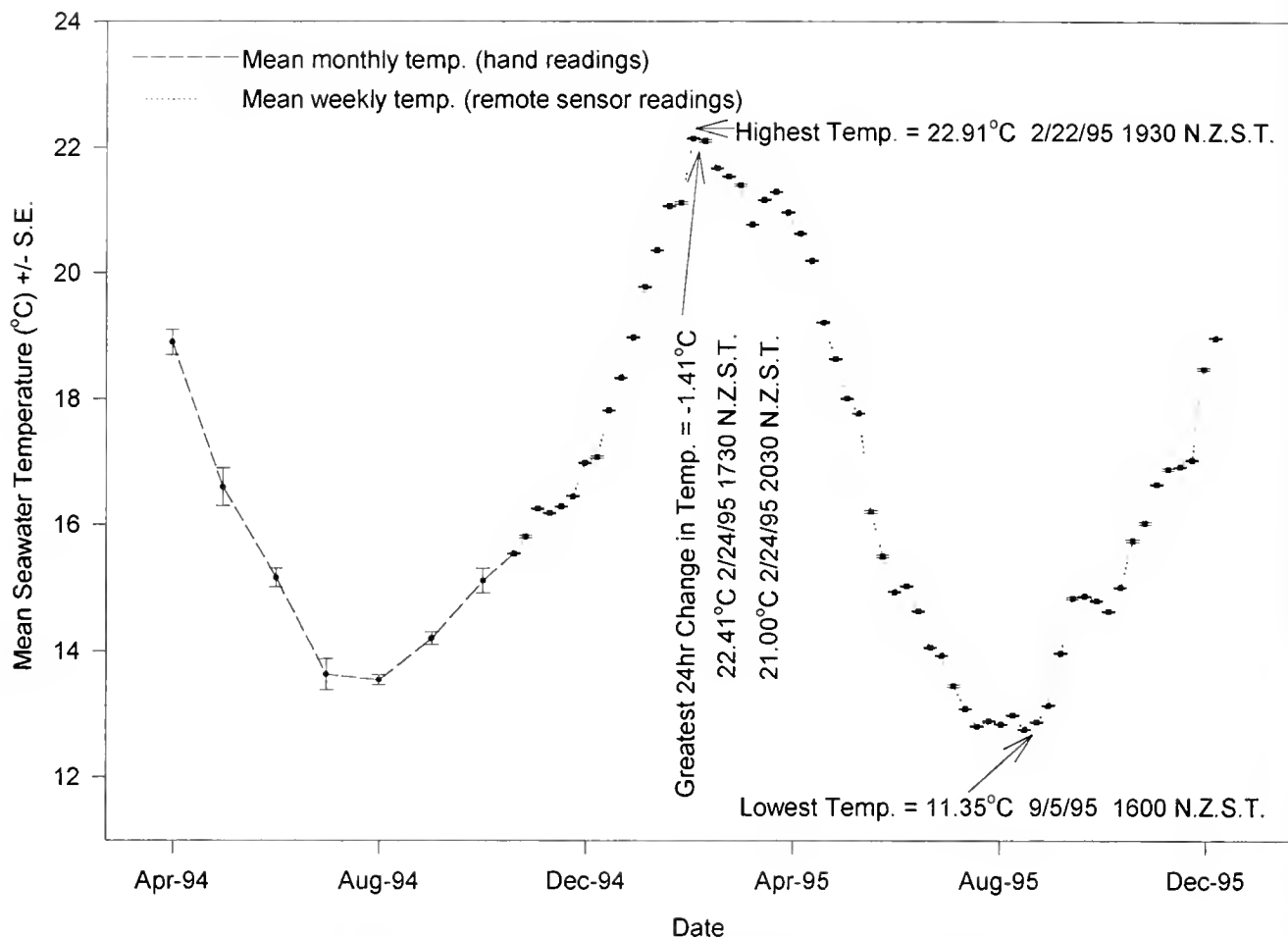


Figure 4. Mean water temperatures (temp.) in the Hauraki Gulf from 1994 to 1995.

of exposure generally occurred in late afternoon and early morning, which were generally the warmest and coolest times of the day. Much smaller daily variations in water temperatures were associated with tidal movements at the Hauraki Gulf study site.

DISCUSSION

Annual Pattern of Larval Settlement

Westerskov (1980) monitored the settlement of *T. chilensis* larvae over 4 y in the Otago Harbour and found that larval settlement began around mid-November, reached a peak in late November to early December, and was finished by February. Although brooding oysters were found outside of these dates, no larval settlement was recorded. Larval settlement in Foveaux Strait followed a similar pattern but later in the year, with 80–90% of spat settling between mid-December and mid-February, whereas some settlement occurred as early as October and some occurred as late as July (Cranfield 1968b, Cranfield 1979). This pattern was confirmed by Stead (1971), although he failed to find any larvae settling between April and August. By comparison, at our two North Island study sites, *T. chilensis* larvae settled throughout the year, with peaks of settlement between late winter and early summer. These patterns of settlement corresponded closely with the annual pattern of larval production, albeit with a delay of 1–2 mo to allow for the completion of brooding and subsequent larval settlement. A delay of a similar period has been found for brooding oysters in Foveaux Strait and Otago Harbour (Cranfield and Allen 1977, Westerskov 1980).

Larval Settlement and Water Temperature

Westerskov (1980) postulated that the timing of both the onset and the peak of spatfall in the Otago Harbour was most closely related to the timing of increases in water temperature after the winter minima of 6–7°C. Larval release and settlement were found to be earlier in those years when the water temperature rose earlier and/or more quickly. The same principle of cumulative temperature exposure has been implicated in the regulation of breeding cycles in other bivalve species (Price and Maurer 1971, Bayne 1975). Westerskov (1980) used data from Cranfield (1968a and b) to demonstrate that the same principle also applied to the Foveaux Strait oyster populations, with an adjustment for the different winter temperature minima (8–11°C). He also used these calculations to explain why the peak of spatfall was consistently later in Foveaux Strait than Otago Harbour, despite sea temperatures in Foveaux Strait showing less seasonal variation than those in the Otago Harbour and remaining several degrees warmer in winter to spring. However, the cumulative temperature exposure principle did not apply to the timing of peak spatfall in the warmer waters of our two study sites at lower latitudes. The annual peak of spatfall was earlier in the Hauraki Gulf than in the Manukau Harbour, despite the Hauraki Gulf showing less seasonal variation in water temperature and remaining several degrees warmer in winter to spring than the Manukau Harbour. Furthermore, the annual peak of spatfall in the Hauraki Gulf coincided with the period of the coldest water temperatures (11.5–13.5°C). Although the exact

mechanism controlling the timing of peak spatfall in this species is not clear, cold seasonal water temperatures has commonly been implicated as the most important cue for synchronizing reproductive activity (Hollis 1962, Westerskov 1980, Winter et al. 1984). For example, at both of our sites, the seasonal peak of settlement was later in 1995 than in preceding year(s), corresponding with the colder water temperatures that were recorded during that winter to spring. Similarly, although larvae were settling at both populations throughout the year, the Manukau Harbour showed a marked peak of settlement that corresponded with the cooler water temperatures in winter. Other populations of *T. chilensis* with colder water temperatures than the Manukau Harbour have been found to have shorter periods of larval production and brief settlement seasons and therefore more pronounced peaks of spatfall (Hollis 1962, Solís 1967, Cranfield 1968a, Cranfield 1968b, Padilla et al. 1969, Stead 1971, Solís 1973, Cranfield and Allen 1977, Osorio 1979, Westerskov 1980, Gleisner 1981, Lépéz 1983, Winter et al. 1983, Winter et al. 1984).

Our findings suggest that cold seasonal water temperatures play an important role in synchronizing larval production and subsequent settlement in *T. chilensis*. At warmer water temperatures (i.e., >12°C), there is less synchronicity in spatfall. There is now a need to experimentally verify the precise nature of the relationship between water temperature and reproduction in *T. chilensis* by attempting to synchronize larval production and spatfall over a range of controlled water temperatures.

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BYSSUS PRODUCTION IN SIX AGE CLASSES OF THE SILVER-LIP PEARL OYSTER, *PINCTADA MAXIMA* (JAMESON)

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ABSTRACT Two experiments were conducted to study byssus production of silver-lip (or gold-lip) pearl oysters, *Pinctada maxima*, from six different age classes. In the first experiment, 75- or 120-day-old *P. maxima* were removed from their point of attachment by severing of the byssus and were placed in clear plastic Petri dishes. The production of byssal threads and the behavior of the pearl oysters were monitored over a 120-h period. Emerging byssal threads were pinkish before changing to green. Juveniles at 75 days of age began reattaching faster than 120-day-old juveniles. However, after the first 12 h, older individuals had produced significantly more ($p < 0.001$) byssal threads than the younger individuals and produced significantly more ($p < 0.001$) byssal threads over the 120-h period. Additionally, byssal thread production for the younger juveniles did not increase significantly ($p > 0.05$) after 48 h, whereas byssal thread production from older animals continued to increase significantly ($p < 0.001$) after this period. The maximum number of threads produced by a single individual in the older age class was 30, compared with 16 in the younger age class. Juvenile *P. maxima* were observed to voluntarily eject the byssal apparatus, move, and reattach within 24 h. Reattachment after voluntary ejection of the byssus was faster than that after mechanical severing. In the second experiment, older *P. maxima* aged 7, 9, 11, or 13 mo were removed from their nets after severing of the byssus with a scalpel. These oysters were placed in nets in an area of either strong (2.5–3.5 knots h^{-1}) or mild (<1 knot h^{-1}) current. Pearl oysters placed in a mild current reattached faster than those in a strong current. However, after 4 days, pearl oysters aged 13 mo in strong current had produced significantly more threads ($p < 0.05$) than those in the mild current, and the same was true for 11-mo-old pearl oysters by Day 5. From Day 5 onward, there were generally more threads produced by pearl oysters in strong current compared with mild current; however, these differences were not significant ($p > 0.05$) for pearl oysters aged 9 and 7 mo. By the end of the 11-day experiment, 9-mo-old oysters had produced significantly more byssal threads than any other age class, and there were significant differences between all age classes in the number of threads produced. The results of these simple experiments provide useful information on the time for reattachment of different age classes of *P. maxima* in a variety of culture conditions after mechanical severing of the byssus.

KEY WORDS: Aquaculture, pearl oysters, *Pinctada*, byssus, attachment

INTRODUCTION

The foot and byssal gland of the silver-lip (or gold-lip) pearl oyster, *Pinctada maxima* (Jameson), provide mobility and anchorage, respectively. The foot, as in all pearl oysters, is a tongue-shaped organ, the bulk of which is a system of multidirectional fibers (Farn 1986). Retractor and levator muscles control foot movement, and extensive blood-filled spaces within the foot provide hydrostatic strength and flexibility (Velayadin and Gandhi 1987). At the proximal end of the foot is the byssal gland, which secretes byssus fibers that pass down a tubular pedal groove (Farn 1986). Muscular contractions of the foot cause the formation of the discoid attachment and stem of each byssal thread. Attachment takes place as the tip of the foot touches the substrate. Byssal secretions harden quickly in seawater, securing the pearl oyster to the substrate (Herdman 1903, Dharmaraj and Alagarswami 1987).

Pinctada fucata (Kafuku and Ikenoue 1983), *Pinctada margaritifera* (Nichols 1931), and *P. maxima* (Saville-Kent 1890, Saville-Kent 1893) juveniles are able to sever their byssal attachment, change position, and reattach. *P. maxima* ceases to use the byssus as a point of anchorage at about 3 y of age, when it is

sufficiently heavy to avoid being moved by ocean currents. However, large (3- to 5-kg) wild *P. maxima* have been found with byssal threads attached to rubble (R.A. Rose, unpubl. data, 1984–1988). In contrast *P. fucata* and *P. margaritifera* maintain byssal attachment as an anchorage system for life (Gervis and Sims 1992).

In aquaculture facilities, regular grading increases growout efficiency by separating faster growers from slower growers and removing individuals that are not growing at a profitable rate. This is particularly important in pearl oyster culture because the timing of the implantation of the pearl nucleus depends on the size of the oyster. As with other byssally attached bivalves, grading requires breaking the byssus to remove animals from their point of attachment (Bourne et al. 1989, Heasman et al. 1994). Generally, the byssus of *P. maxima* is severed with a scalpel or razor blade before grading. For commercial rearing of *P. maxima*, the period required for reestablishment of the byssus is important because of the common practice of using pressurized water for routine cleaning of pearl oysters. If pearl oysters have not reestablished a firm anchorage, this method of cleaning may prove harmful or even fatal.

P. maxima growers recognize weak byssal attachment or detachment by juvenile pearl oysters as a sign of ill health (J. Jorgensen, M. Pieper, and S. Arrow, pers. comm., 1993–1997). Detachment, accompanied by other symptoms such as mantle retraction, has been observed before and during mass mortality incidents

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(losses of up to 75% of the population) in juvenile *P. maxima* (J.J. Taylor and R.A. Rose, unpubl. data, 1993–1997). Knowing how long it takes for the byssus to regenerate and if the time required varies as pearl oysters grow would therefore aid growout management and provide valuable data for general health monitoring in commercial operations. To this end, this study determined the time required for reattachment for six age classes of *P. maxima* juveniles, after mechanical severing of the byssus.

MATERIALS AND METHODS

Experiment 1

Plastic Petri dishes were used as experimental settlement substrata. The Petri dishes provided a clear substrate through which attachment was observed and individual byssal threads could be counted over time. Byssus were observed by inverting the Petri dishes under a dissecting microscope. Juveniles of two age classes were used: 75-day-old juveniles, with mean (\pm SE) dorsoventral shell height and anteroposterior length of 6.7 ± 0.5 and 10.9 ± 1.0 mm, respectively, and 120-day-old juveniles, with mean (\pm SE) shell height and length of 14.9 ± 1 and 20.7 ± 1.4 mm, respectively. Fourteen juveniles of each age class were used for the experiment. At Time 0, juveniles were removed from their point of attachment by severing of the byssus with a scalpel blade. Two juveniles of the same age class were placed in each of 14 Petri dishes. Juveniles were monitored every 30 min for the first 3 h. Juveniles were inspected for the following 6 days, and attached byssal threads were counted.

Experiment 2

This experiment was conducted with pearl oysters of 7, 9, 11, and 13 mo of age. Before the start of the experiment, the number of byssal threads of 25 randomly selected individuals from each age class was counted before the oysters were removed from their point of attachment. The same animals were also measured and weighed. Oysters of 12 mo of age were held in 8-pocket panel nets (Gervis and Sims 1992), while all other age classes were held in 28-pocket panel nets. Only 10 pearl oysters were placed in each net, and 16 nets were used for each age class. Half of the pearl oysters from each age class were placed in an area of either strong current (2.5–3.5 knots h^{-1}) or mild current (<1 knot h^{-1}) near the island of Bacan, Maluku Utara, Indonesia (lat. 1°S , long. 127°E). The number of byssal threads produced by each pearl oyster was counted on Days 1 to 7 after the start of the experiment and again on Days 9 and 11.

Statistical Analysis

Data were compared using analysis of variance (Sokal and Rohlf 1981); means were compared using Fisher's Protected Least Significant Difference test from the StatView statistical program, version 4.5, for Macintosh computers (Abacus Concepts, StatView 1992). Homogeneity of variances was confirmed using Cochran's test (Snedcore and Cochran 1967).

RESULTS

Experiment 1

Figure 1 shows the number of byssal threads produced by pearl oysters, in each of the two age classes, at intervals during the 120-h experiment. After only 2 h, 6 of the 14 younger individuals (75

days old) were able to hold position when inverted and washed gently with seawater. However, no byssal threads were evident at this time and position appeared to be maintained by the foot alone. Younger individuals showed significantly greater byssal thread production than older individuals (120 days old) during the first 3 h of the experiment ($p < 0.001$; Fig. 1). Within 12 h, all pearl oysters, in both age classes, had formed a byssal attachment. After 12 h, older individuals had produced significantly more byssal threads than younger individuals ($p < 0.001$; Fig. 1). The total number of threads produced over the 120-h period also differed significantly ($p < 0.001$); the older pearl oysters produced 21.9 ± 1.1 threads (mean \pm SE), and the younger pearl oysters produced 11.3 ± 0.8 threads. Byssal thread production for the younger juveniles did not increase significantly ($p > 0.05$) after 48 h, whereas older individuals produced significantly more threads each day from 24 h onward ($p < 0.001$).

New and emerging byssal threads appeared pinkish. Within a few hours, they began to change color, initially becoming translucent before gaining a greenish hue. The color darkened and the threads thickened over time. The point of attachment was splayed (Figs. 2 and 3), and the fibers of the byssal threads were obvious at the point of attachment. On flat surfaces, byssal threads were arranged in a radial pattern (Fig. 2). Where juveniles had moved to the edge of the Petri dish and attached to the dish wall, the threads were attached predominantly in a single direction (Fig. 3).

In many instances, byssal threads were ejected from the byssal gland and were observed with one end still attached to the Petri dish and the other end floating free (Fig. 4). In some cases, the entire byssus was jettisoned and the oysters had moved some distance before reattaching. This loss and replacement of byssal threads occurred within 24 h.

Experiment 2

The mean (\pm SE; $n = 25$) shell length, shell height, and wet weight (WW) and the number of byssal threads (BT) for each age class at the start of the experiment are shown in Figure 5. The 13-mo-old *P. maxima* had significantly fewer ($p < 0.01$) byssal threads (8.9 ± 0.7) than did any other age class. The number of byssal threads counted from 11-mo-old *P. maxima* did not differ significantly ($p > 0.05$) from those from the 9- or 7- mo-old individuals, but the 7-mo-old individuals had significantly fewer byssal threads ($p < 0.05$) than did the 9-mo-old oysters (Fig. 5). Significant differences resulted when the ratios of WW to BT were compared (Fig. 6). The WW/BT ratio for 13-mo-old individuals

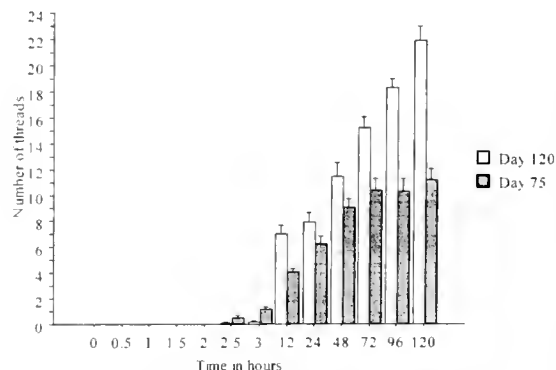


Figure 1. Byssus thread production over time (mean \pm SE; $n = 14$) in 75- and 120-day-old *P. maxima* juveniles.

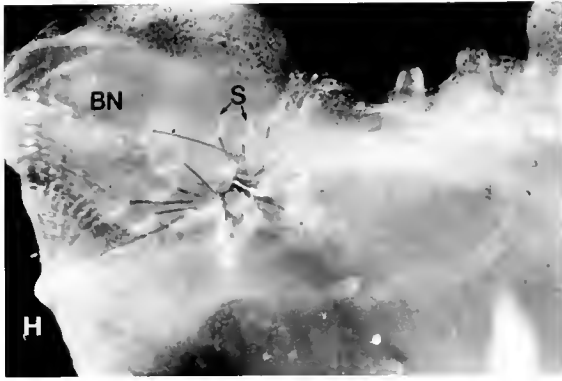


Figure 2. Byssal threads of a juvenile *P. maxima* attached to the flat surface of a Petri dish and arranged in a radial pattern. This juvenile attached to the flat surface in the center of a Petri dish. Note: H, the hinge of the oyster; BN, the byssal notch; S, the splayed end of the byssal threads at the point of attachment.

was significantly greater ($P < 0.01$) than that for any other age class. The WW/BT ratio became significantly less ($P < 0.01$) with each age class, with the exception of the 9- and 7-mo-old pearl oysters, where the WW/BT ratio did not differ significantly ($p > 0.05$).

After mechanical severing of the byssal threads, differences in byssus production were noted due to both age and current strength. Regardless of age, significantly more byssal threads were produced by oysters in the mild current area during the first day of the experiment (Table 1). This was also true after Day 2 for all but the 13-mo-old oysters. From Day 5 onward, there were generally more threads produced by pearl oysters in strong current compared with mild current; however, these differences were not significant ($P > 0.05$) for pearl oysters aged 9 and 7 mo. By the end of the experiment, pearl oysters aged 9 mo had produced significantly more threads ($p < 0.01$) than any other age class and differences in the number of threads produced were significant ($p < 0.01$) between all age classes.

A number of individuals from each age class ejected the original byssal plug from the shell cavity. After 11 days, no oyster in any of the age classes had produced the number of threads that

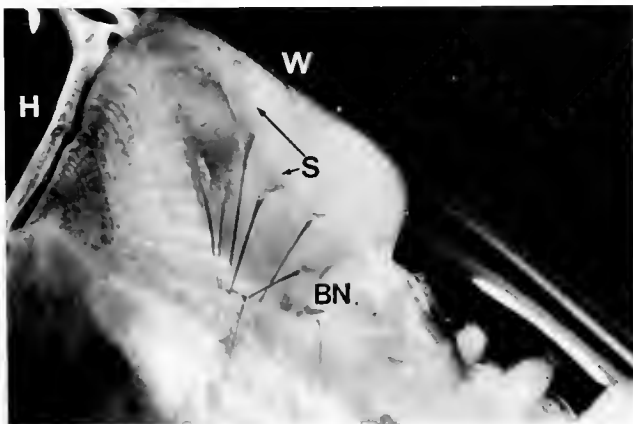


Figure 3. Byssal threads of a juvenile *P. maxima* attached near to the wall of a Petri dish with the threads predominantly in a single direction. Note: H, the hinge of the oyster; BN, the byssal notch; S, the splayed end of the byssal thread at the point of attachment; W, the wall of the Petri dish.

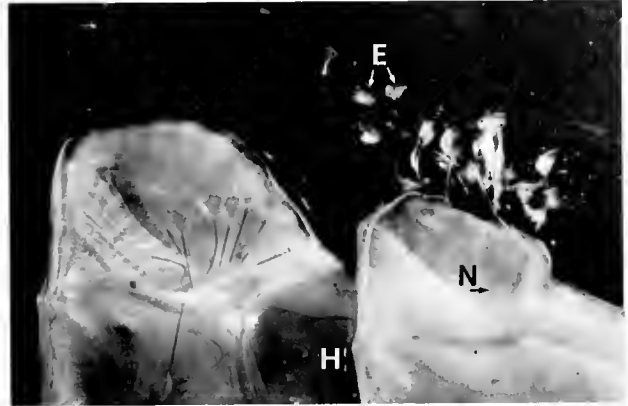


Figure 4. A juvenile *P. maxima* (far right) that has detached, changed position, and reattached, leaving ejected byssal threads behind. Note: H, the hinge of the oyster; N, new byssal threads; E, ejected byssal threads that are still attached to the surface of a Petri dish.

were counted at the start of the experiment and some of the older individuals did not reattach at all.

DISCUSSION

Juveniles of 75 days of age began reattaching faster than 120-day-old juveniles. However, after the first 12 h, older *P. maxima* produced significantly more threads than the younger individuals and significantly more threads over the 120-h experiment. Moreover, byssal thread production for the younger juveniles did not increase significantly after 48 h, whereas production from older animals continued to increase significantly. This suggests that younger pearl oysters regain maximal anchorage after a shorter period than older pearl oysters. The maximum number of threads produced by a single individual in the older age class was 30 compared with 16 in the younger age class. A stronger anchorage may have been required by the older individuals to compensate for greater resistance to water currents due to larger surface area. Saville-Kent (1890, 1893) reported that juvenile *P. maxima* of a size range between 8 and 65 mm had 30–40 byssal threads. Rose and Baker (1994) reported the average number of byssal threads in 10- to 15-mm *P. maxima* juveniles to be approximately 20; neither study reported differences in byssal production with age or between size classes.

Juvenile *P. maxima* have the ability to sever the byssus, move, and reattach (Saville-Kent 1890, 1893). This behavior was observed in this study with juveniles moving and reattaching with the same or a greater number of threads within a 24-h period. One

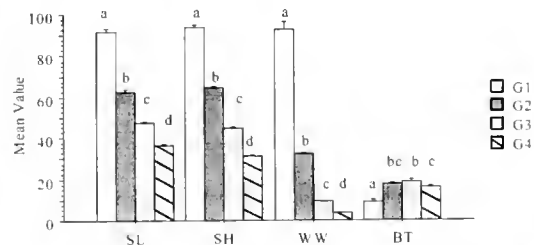


Figure 5. Mean (\pm SE) shell height (SH), shell length (SL), wet weight (WW), and number of byssal threads (BT) of four age classes of *P. maxima*. G1, 13 mo old; G2, 11 mo old; G3, 9 mo old; G4, 7 mo old. Values with the same superscript for each variable are not significantly different ($p > 0.05$).

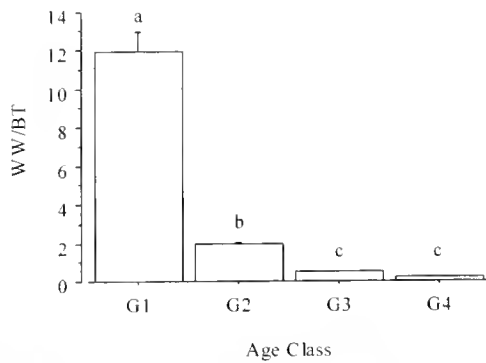


Figure 6. Mean (\pm SE) ratio of wet weight (WW) to number of byssal threads (BT) of four age classes of *P. maxima*. G1, 13 mo old; G2, 11 mo old; G3, 9 mo old; G4, 7 mo old. Values with the same superscript for each variable are not significantly different ($p > 0.05$).

younger individual moved twice within a 24-h period and produced a total of 20 new byssal threads. This suggests that when a pearl oyster voluntarily ejects the byssus, it can reattach more rapidly than after mechanical severing. *P. maxima* juveniles can also eject individual threads, which will allow minor positional changes, perhaps to adjust to water flow without losing the security of the entire byssus.

The number of byssal threads counted at the start of the second experiment shows the reduction in byssal thread production as *P. maxima* ages. Presumably, a point is reached where the increased water resistance, due to greater surface area, is offset by the greater stability resulting from increased mass. Eventually, the weight of the pearl oyster is such that byssal attachment loses importance as the main means of maintaining position. The large differences in the ratio of wet weight to the number of byssal threads supports this notion. Even in very large specimens of *P. maxima* (>1 kg WW), the byssus may still be observed, even though there is no attachment to substrata and therefore no byssal anchorage (R.A. Rose, unpublished data 1984–1988).

In the second experiment, *P. maxima* initially reattached with a greater number of threads when placed in relatively calm water with little current. It appears that stronger current made initial reattachment more difficult. This may be because the net holding the oysters was less stable under these conditions. After initial attachment, greater numbers of threads were produced by pearl oysters in the strong current, indicating that additional threads were required to secure the pearl oysters in the nets. This was particularly the case for the oldest and largest individuals, which in some cases, failed to reattach during the 11-day experiment. The results of this study indicate that byssus production in *P. maxima*

TABLE 1.

Mean (\pm SE) byssal thread production of four age classes of silver-lip pearl oyster, *P. maxima*, placed in an area of either strong current (SC) or mild current (MC).

Day	Current	G1	G2	G3	G4
1	SC	0.2 \pm 0.1 ^{a1}	0.5 \pm 0.1 ^{b1}	1.1 \pm 0.1 ^{c1}	1.3 \pm 0.1 ^{c1}
	MC	0.5 \pm 0.1 ^{a2}	0.9 \pm .01 ^{b2}	1.8 \pm 0.1 ^{c2}	2.1 \pm 0.1 ^{c2}
2	SC	1.0 \pm 0.2 ^{a1}	1.1 \pm 0.2 ^{b1}	2.6 \pm 0.2 ^{c1}	2.9 \pm 0.2 ^{c1}
	MC	1.1 \pm 0.2 ^{a1}	1.9 \pm 0.2 ^{b2}	3.3 \pm 0.2 ^{c2}	3.3 \pm 0.2 ^{c2}
3	SC	1.8 \pm 0.2 ^{a1}	2.2 \pm 0.2 ^{b1}	4.3 \pm 0.3 ^{c1}	4.5 \pm 0.2 ^{c1}
	MC	1.5 \pm 0.2 ^{a1}	2.4 \pm 0.2 ^{b1}	4.1 \pm 0.3 ^{c1}	4.5 \pm 0.2 ^{c1}
4	SC	2.7 \pm 0.3 ^{a1}	3.1 \pm 0.3 ^{b1}	5.6 \pm 0.3 ^{c1}	5.7 \pm 0.2 ^{c1}
	MC	1.9 \pm 0.3 ^{a2}	3.1 \pm 0.3 ^{b1}	5.4 \pm 0.3 ^{c1}	6.0 \pm 0.3 ^{c1}
5	SC	3.8 \pm 0.4 ^{a1}	4.9 \pm 0.3 ^{b1}	7.1 \pm 0.4 ^{c1}	7.1 \pm 0.3 ^{c1}
	MC	2.5 \pm 0.4 ^{a2}	3.6 \pm 0.3 ^{b2}	7.2 \pm 0.4 ^{c1}	6.8 \pm 0.3 ^{c1}
6	SC	4.8 \pm 0.4 ^{a1}	5.6 \pm 0.4 ^{b1}	8.7 \pm 0.4 ^{c1}	8.6 \pm 0.4 ^{c1}
	MC	3.0 \pm 0.4 ^{a2}	4.5 \pm 0.3 ^{b2}	8.2 \pm 0.4 ^{c1}	8.1 \pm 0.3 ^{c1}
7	SC	5.0 \pm 0.4 ^{a1}	6.4 \pm 0.4 ^{b1}	9.7 \pm 0.4 ^{c1}	8.7 \pm 0.3 ^{d1}
	MC	3.5 \pm 0.4 ^{a2}	5.9 \pm 0.3 ^{b1}	9.6 \pm 0.4 ^{c1}	8.6 \pm 0.4 ^{d1}
9	SC	7.0 \pm 0.4 ^{a1}	8.6 \pm 0.4 ^{b1}	11.9 \pm 0.5 ^{c1}	9.8 \pm 0.4 ^{d1}
	MC	5.0 \pm 0.4 ^{a2}	7.4 \pm 0.4 ^{b2}	11.2 \pm 0.5 ^{c1}	10.1 \pm 0.4 ^{d1}
11	SC	7.8 \pm 0.4 ^{a1}	9.8 \pm 0.5 ^{b1}	13.0 \pm 0.6 ^{c1}	11.0 \pm 0.4 ^{d1}
	MC	6.2 \pm 0.5 ^{a2}	9.0 \pm 0.4 ^{b2}	12.0 \pm 0.5 ^{c1}	10.1 \pm 0.4 ^{d1}

G1, pearl oysters aged 13 mo; G2, pearl oysters aged 11 mo; G3, pearl oysters aged 9 mo and G4, pearl oysters aged 7 mo. Means with the same superscript (alphabetical across rows, numerical down columns) are not significantly different ($p > 0.05$).

may be the result of a subtle relationship between the stability of the substratum, the resistance of a pearl oyster to a given current, and the size and weight of the individual. In this experiment, the pearl oysters were maintained under typical farm conditions—vertically orientated in panel nets. The results may have differed had the pearl oysters been placed flat on the seabed, where resistance to the current would have been reduced.

The results of these simple experiments provide useful information on the time required for reattachment after mechanical severing of the byssus of *P. maxima*. Where possible, it is advised that newly graded pearl oysters, or oysters that have been transferred to new nets, should be placed in areas with calm water for a minimum of 24 h to allow a reasonable degree of reattachment before moving them into areas of higher current or wave action.

ACKNOWLEDGMENTS

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BREEDING CYCLE OF PEARL OYSTERS *Pinctada mazatlanica* AND *Pteria sterna* (BIVALVIA:PTERIIDAE) AT BAHIA DE LA PAZ, BAJA CALIFORNIA SUR, MEXICO

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ABSTRACT The breeding cycles of pearl oysters *Pinctada mazatlanica* and *Pteria sterna* were studied from June 1992 to May 1993 as part of a Pearl Culture Program in Bahía de La Paz. Gonad samples of 20 oysters of each species were collected monthly (480 over the annual cycle) and processed for histological examination. We studied the annual breeding cycle of both species, the sex ratio as a function of time, and the size of the oysters. The results obtained by histological analysis were confirmed by similar changes in a gonadosomatic index. Gametogenesis was continuous throughout the year in both species. *P. mazatlanica* spawned once a year (September), when water temperature reached 29.5°C. It is a protandrous hermaphrodite in which sex reversal was observed in oysters larger than 100-mm shell height. The female:male sex ratio was 0.12:1. Gonad maturity was found in oysters larger than 39 mm. *P. sterna* spawned twice a year (February and May), when water temperature was 22.2 and 23.4°C. There was not enough evidence to conclude that *P. sterna* was a protandrous hermaphrodite. If that were the case, sex reversal would have occurred in oysters larger than 50-mm shell height. The female:male sex ratio was 0.38:1. Gonad maturity was seen in oysters larger than 40 mm.

KEY WORDS: Pearl oysters, breeding cycle, reproduction, repopulation, Bahía de La Paz

INTRODUCTION

In México, natural populations of the native species *Pinctada mazatlanica* (Hanley, 1856) and *Pteria sterna* (Gould, 1851) are now in a critical situation because of overexploitation. The uncontrolled pearl fishery carried out in Bahía de La Paz for more than 400 years depleted the natural stocks along the coast almost entirely by 1940 (Sevilla 1969, Shirai and Sano 1979, Cariño 1987, Cariño and Cáceres-Martínez 1990, Monteforte 1990, Monteforte 1991, Monteforte and Cariño 1992, Cariño and Monteforte 1995). At present, both species are under a legal ban decreed on the pearl oyster fishery (Diario Oficial de la Federación 1939) and are considered "species under special protection" (Diario Oficial de la Federación 1994). However, illegal extractions have continued, impeding the natural recovery of broodstock.

The presence of pearl oysters in the Baja California Península has played an important role in the social and economic development of the region, mainly in Bahía de La Paz. Therefore, the urgent need to apply strategies for conservation, extensive culture, repopulation, and recovery of the nacre resource has been emphasized on several occasions (Sevilla 1969, Díaz-Garcés 1972, Martínez 1983, Monteforte 1990, Monteforte 1991, Monteforte and Cariño 1992, Saucedo and Monteforte 1994, Saucedo et al. 1994).

The success of aquaculture of pearl oysters requires a proper knowledge of the biology and ecology of the species. To understand the population dynamics of the wild stock and, more recently, the development of pearl-culture strategies, it is essential to understand the reproductive biology of pearl oysters (Tranter 1958a, Sevilla 1969, Rose et al. 1990, García-Domínguez et al. 1996).

There have been a number of studies of the reproductive biology of the genus *Pinctada*. These studies reveal that most aspects of the breeding cycle of pearl oysters are common to all species. They seem to be functional protandrous hermaphrodites (maturing as males and changing to females at a certain size, regulated by

internal and external processes). The ratio of males to females tends toward 1:1 with increasing age (Gervis and Sims 1992). However, little is known about the reproductive biology of *P. mazatlanica* and *P. sterna*. Before this study, the only data available were those of Sevilla (1969), who made a precise microscopic description of the gonad anatomy of *P. mazatlanica*, pointing out each phase of the breeding cycle and its seasonal occurrence. No study has been done on *P. sterna*.

In 1987, we started an applied research program on pearl oyster culture and pearl induction at the Centro de Investigaciones Biológicas del Noroeste, in Bahía de La Paz. Parallel to technological development aiming at production through extensive culture and induction to pearl formation, we are also searching for recovery and conservation strategies. The objective of this study is to describe the annual breeding cycle of the pearl oysters *P. mazatlanica* and *P. sterna*, obtained from extensive culture and kept under bottom-culture conditions in Bahía de La Paz.

MATERIALS AND METHODS

Oysters used in this study were collected in 1991 at Isla Gaviota and reared by extensive culture at Caleta El Merito, located on the southwest coast of Bahía de La Paz, between 24°46' and 24°07'N, and 110°38' and 110°18'W (Fig. 1).

The selection of Caleta El Merito as the study area was made because of its climatic, geomorphologic, and oceanographic conditions, which were adequate for the development of the study. A more detailed description of the area is provided in Alvarez-Borrego and Schwartzlose (1979), Osuna-Valdéz (1986), Murillo (1987), and Monteforte and Cariño (1992).

In April 1992, 480 oysters (240 of each species) were transferred to bottom-culture conditions, using plastic pearl cages (70-cm length, 40-cm width, and 20-cm height) placed on a submerged shelf at 10-m depth. Sixteen cages were placed on the bottom of the study area (eight per species), each one containing 30 oysters. The initial size range varied from 39.5 to 136.5-mm shell height for *P. mazatlanica* (mean, 80.55; SD, 20.25) and from 41.1 to 89.2 mm for *P. sterna* (mean, 66.12; SD, 12.30).

Twenty oysters of each species were collected monthly using

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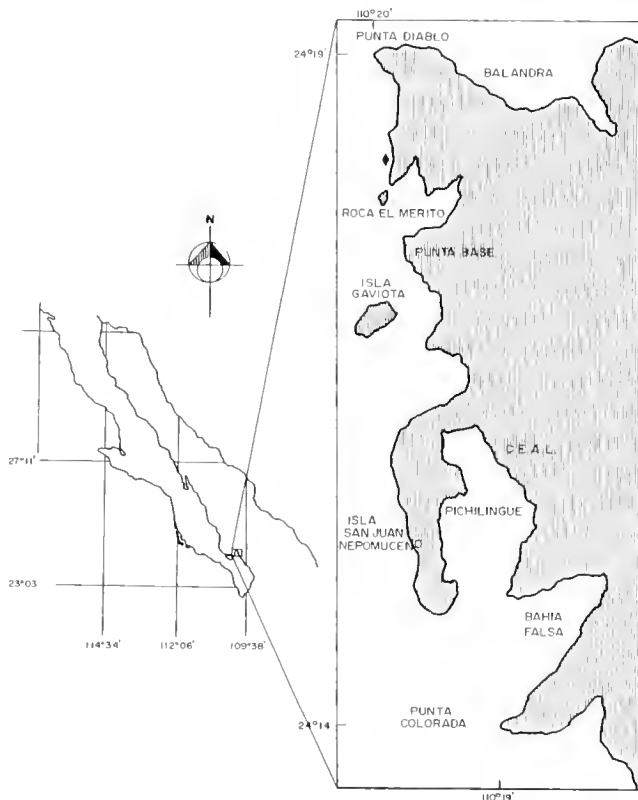


Figure 1. Location of the study area (Caleta El Merito) inside Bahía de La Paz, indicated by closed diamond.

SCUBA gear, and they were preserved in 10% formalin for 48 h. Before dissection, the following shell measurements were taken with plastic calipers (± 0.01 mm) according to Hynd's expressions (1955): height or dorsoventral measurement, length or anteroposterior measurement, thickness, wet weight of the oyster with shell, wet weight without shell, and wet weight of the visceral mass in which the gonadal tissue is intermingled. This latter sample, always excised between the labial palps, near the foot, and the intestine tube, was processed for histological examination. Samples were embedded in paraffin, sectioned at 7 or 8 μ m, and stained by the hematoxylin-eosin technique. They were analyzed with a compound microscope at low magnifications (10 \times and 40 \times) and were photographed through the microscope.

To analyze the breeding cycle of both species, and especially to understand the seasonal changes occurring in the gonads, we used five broad gametogenic stages, using the schemes developed by Sevilla (1969) for *P. mazatlanica*, and Rose et al. (1990) for *P. maxima*. The stages are: (1) indeterminate or inactive, (2) developing or near-ripe, (3) maturity or ripe, (4) spawning, and (5) spent.

We also calculated the total female:male sex ratio of both species, the sex ratio as a function of time, and the sex ratio related to the size of the oysters. Shell height was used as the most adequate indicator of growth. It is considered the largest dimension of the oyster measured at right angles to the hinge line, excluding the growth processes (Hynd 1955).

At the same time as histological analysis was done, a gonadosomatic index (GI) was calculated with the oyster's measurements originally taken, using the equation proposed by Sastry (1970):

$$GI = GW / WWS \times 100$$

This is obtained by dividing the gonad weight of the animal (GW) by its wet weight without shell (WWS), multiplied by 100. Finally, the relationship between the GI and the monthly changes in the water temperature during the annual cycle was also studied.

RESULTS

Gonad Developmental Stages

Gametogenesis was found to be a continuous process throughout the annual cycle in both species. However, many of the stages of the breeding cycle overlapped in time within the same gonad, so their classification into any gametogenic stage was sometimes difficult to determine. The most important microscopic characteristics of the gonad anatomy are described as follows:

Indeterminate or Inactive

There is no evidence of gonad development. Instead, the gonad consists mainly of connective tissue. Follicles are completely empty and may contain some phagocytes. Gonads are not able to be classified as to sex (Fig. 2A).

Developing or Near-Ripe

The production of gametes begins. At first, follicles are small and poorly developed. Oogonia in the ovary and spermatogonia in the testis are mainly connected to the follicular wall (Figs. 2B and 3A). As gametogenesis proceeds, different stages of gametes can be observed. In the testis, primary and secondary spermatocytes proliferate rapidly. In the ovary, connected and some free oocytes with little or no yolk expand into the lumen (Figs. 2C and 3A). At the final stages of gametogenesis, spermatids and some spermatozoa, or free oocytes with yolk and nucleolus, are common in the follicles. During this stage, the amount of connective tissue rapidly decreases and almost disappears.

Maturity or Ripe

The gonad has grown and enlarged as a compact and uniform mass, in which the individual follicles are distended and hard to distinguish. Connective tissue has been reduced to a small and thin layer in the distal regions of the gonad. The follicular lumen is filled mainly with polygonal-shaped free oocytes with yolk and nucleolus (in the ovary) or with spermatozoa clearly defined by their eosinophilic tails (in the testis). Some isolated pockets of developing oogonia or spermatids can be observed (Figs. 2D and 3C).

Spawning

This phase is easy to detect because of the expulsion of gametes. Follicles are broken, distended, and partially empty. The lumen is filled with residual free oocytes or thin spermatozoa, showing signs of regression (Figs. 2E and 3D).

Spent

Follicles have become extremely thin, and the lumen is practically empty, with some isolated pockets of residual oocytes or spermatozoa. This phase is characterized by the rapid proliferation

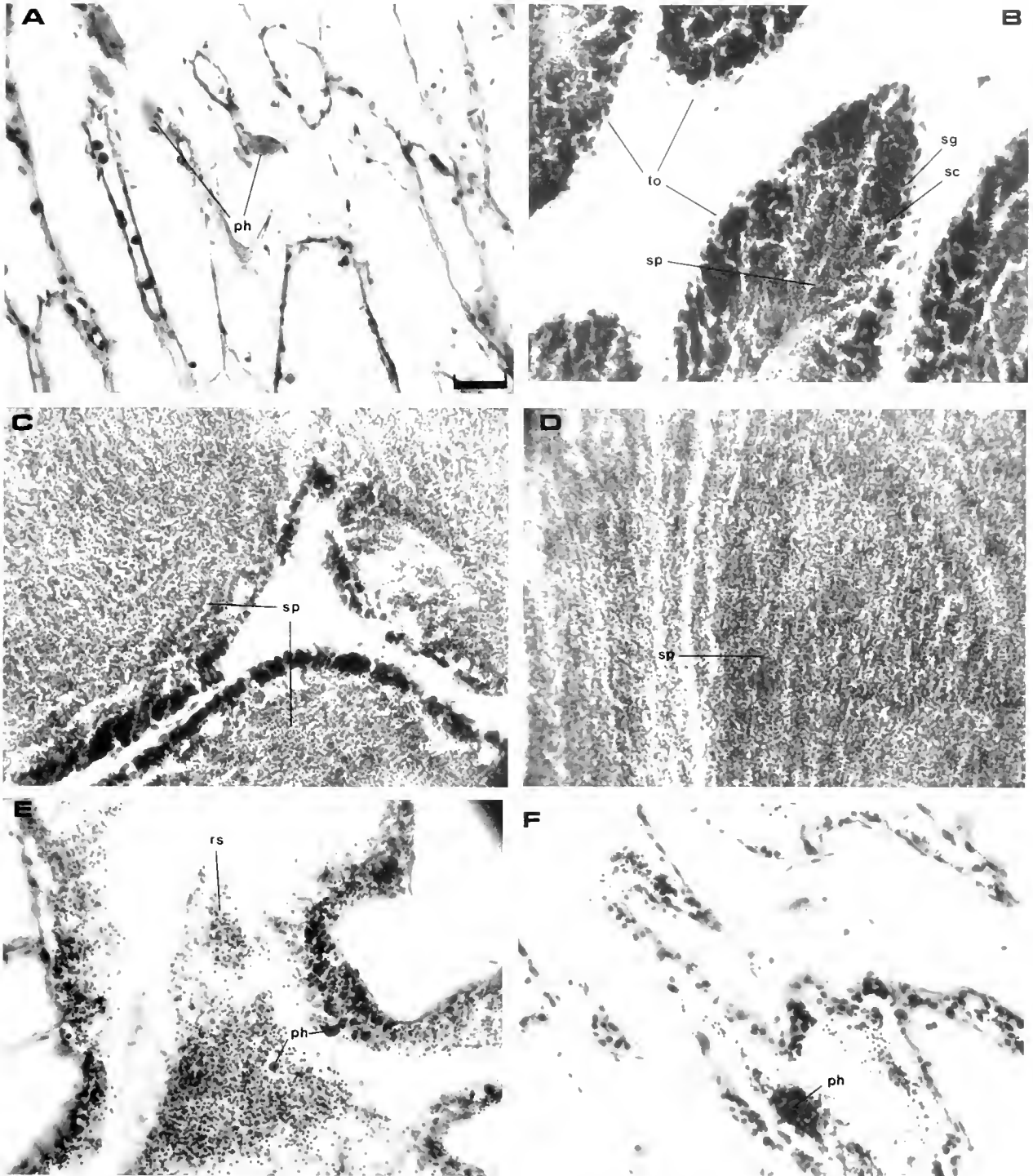


Figure 2. Sexual phases of male gametogenesis in *P. mazatlanica* and *P. sterna*. (A) Indeterminate phase in *P. sterna*, showing empty follicles with some phagocytes (ph); (B) early gametogenesis in *P. sterna*, in which small follicles (fo) contain spermatogonia (sg) connected to the follicular wall, primary and secondary spermatocytes (sc) expanding toward the lumen, and some spermatozoa (sp) filling the center; (C) advanced gametogenesis in *P. mazatlanica* with distended follicles containing large amounts of spermatozoa; (D) maturity stage in *P. mazatlanica*, characterized by the presence of follicles packed with spermatozoa almost exclusively; (E) spawning in *P. mazatlanica* with broken and partially empty follicles containing residual spermatozoa (rs), and the presence of different kinds of phagocytes; (F) spent stage in *P. sterna*, in which empty and collapsed follicles contain high phagocytic activity; connective tissue is developing again. Scale bar, 25 μ m.

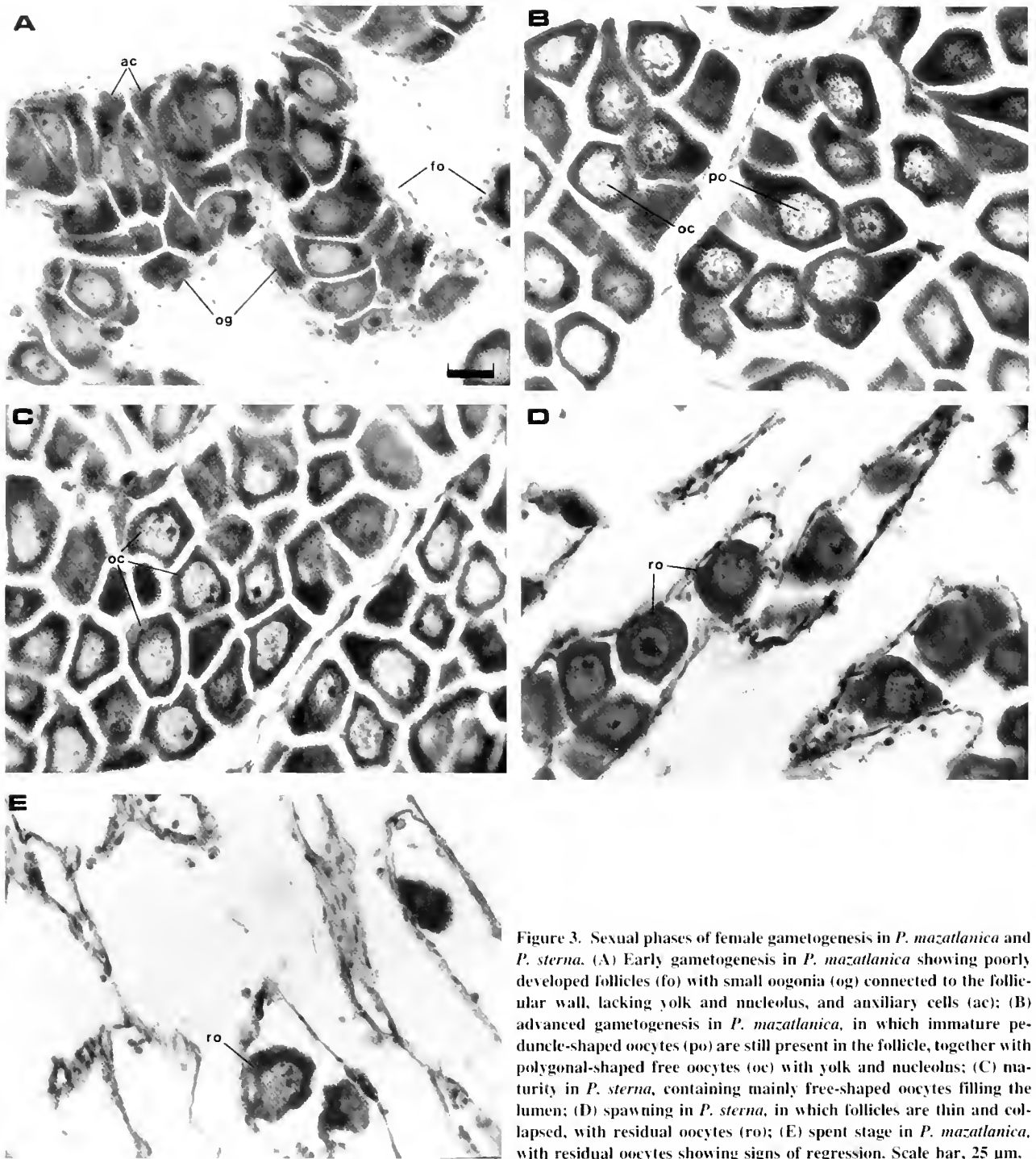


Figure 3. Sexual phases of female gametogenesis in *P. mazatlanica* and *P. sterna*. (A) Early gametogenesis in *P. mazatlanica* showing poorly developed follicles (fo) with small oogonia (og) connected to the follicular wall, lacking yolk and nucleolus, and auxillary cells (ac); (B) advanced gametogenesis in *P. mazatlanica*, in which immature peduncle-shaped oocytes (po) are still present in the follicle, together with polygonal-shaped free oocytes (oc) with yolk and nucleolus; (C) maturity in *P. sterna*, containing mainly free-shaped oocytes filling the lumen; (D) spawning in *P. sterna*, in which follicles are thin and collapsed, with residual oocytes (ro); (E) spent stage in *P. mazatlanica*, with residual oocytes showing signs of regression. Scale bar, 25 μ m.

of different kinds of phagocytes surrounding the gametes. The connective tissue has started to develop again (Figs. 2F and 3E).

P. mazatlanica

Breeding Cycle

The breeding cycle of *P. mazatlanica* is shown in Figure 4. In June and July, oysters at different developmental stages are common; a large number of them have started gonad development, others have reached maturity, and another group was found to be spent. Spawning took place in September and October, and most

oysters were spent in October and November. Gonad development continued in November and lasted until May. Mature oysters were seen from February to May.

Sex Ratio

This was completely skewed to the male sex. From the total sample analyzed, 77% were male, 9% female, and the last 13% were indeterminate. The female:male sex ratio was 0.12:1.

The sex ratio related to oyster size revealed that *P. mazatlanica* matured as male and tended to be a protandrous hermaphrodite.

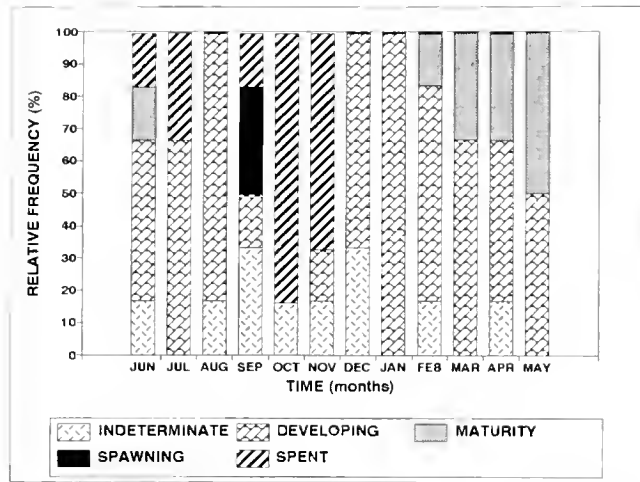


Figure 4. Sexual gametogenic stages in *P. mazatlanica* during an annual cycle.

This is because males were seen from 40 to 150-mm shell height. Females, on the other hand, never appeared under 100 mm. Indeterminate oysters were observed from 40 to 80 mm (Fig. 5).

Figure 6 shows the percentage of females and males as a function of time. Once again, males were present during the entire annual cycle, but with a higher incidence in January, February, and April, months in which gonads were in active development or ripe. Females appeared only from June to August and from February to May, again, the months with greatest reproductive activity.

GI

This index was found to be a good indicator of the reproductive activity of the animals because it revealed a close relationship with the reproductive activity of the oysters (Fig. 7). The highest values of the index denoted an increase in the reproductive activity, and gonads were found in active development or mature (July 1992 and from January to May 1993). The lowest value, in September, coincided with the spawning. Figure 7 also shows the relationship between the GI and the water temperature. Once again, in September, when the water temperature was 29.5°C, spawning oc-

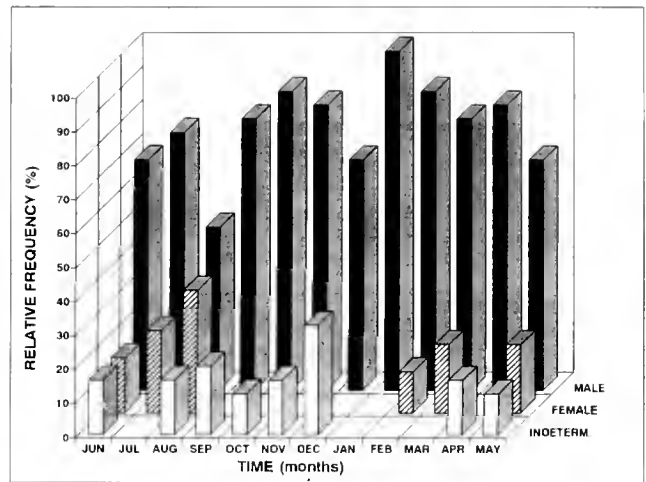


Figure 6. Temporal sex ratio in *P. mazatlanica*.

curred. After December, increasing water temperature resulted in gradual increases in the values of the GI.

P. sterna

Breeding Cycle

The breeding cycle of *P. sterna* is shown in Figure 8. Some gonad development can be detected in June. In July, oysters were spent, although the largest part of the sample was found to be indeterminate. This stage was present almost all year. Gametogenesis was seen continuously from August 1992 to May 1993. Spawning took place in February. We detected a new, short breeding cycle, including gametogenesis, maturity, and a second spawning in May.

Sex Ratio

Once again, the sex ratio was skewed to the male sex. From the total sample, 48% were male, 19% were female, 0.6% were hermaphrodite, and 32% were indeterminate. The female:male sex ratio was 0.38:1.

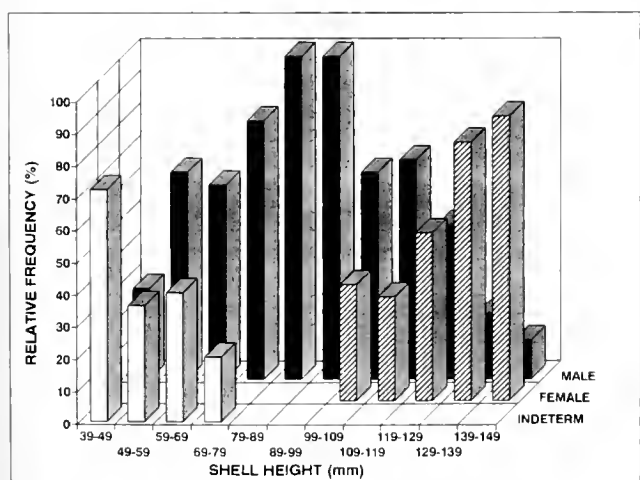


Figure 5. Size-related sex ratio in *P. mazatlanica*.

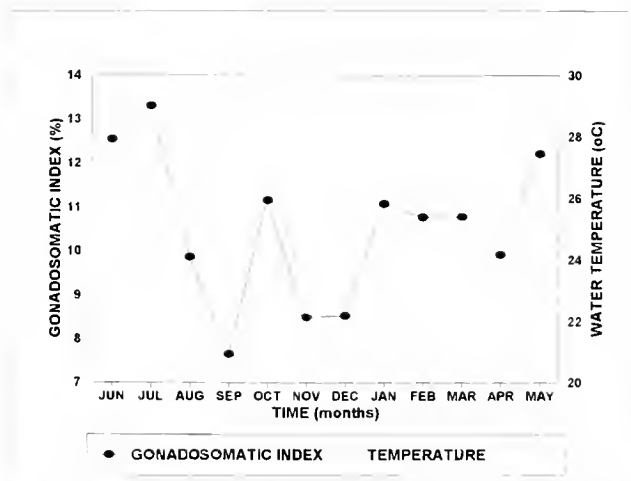


Figure 7. Relationship between the GI and the water temperature for *P. mazatlanica*.

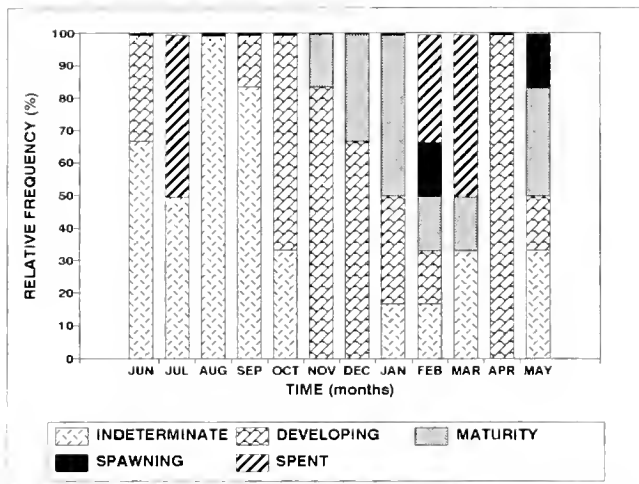


Figure 8. Sexual gametogenic stages in *P. sterna* during an annual cycle.

Figure 9 shows the percentage of females and males. The size-range analysis suggests that *P. sterna* can be a protandrous hermaphrodite. Males were present from 40 to 85-mm shell height, with a higher incidence between 40 and 55 mm. Females appeared after 50–55 mm but were not represented in all of the size ranges. Their maximum number was observed between 60 and 65 mm and 85 and 90 mm. Indeterminate oysters were present in all of the size ranges (Fig. 9).

Figure 10 shows the sex ratio as a function of time. Once again, the male sex was present over the entire annual cycle, but with a higher incidence in January, February, and April during the spawnings. Females appeared from August on and were present the rest of the annual cycle. This behavior, unlike that of *P. mazatlanica*, could indicate that this species is a multispawner.

GI

The index seems to describe adequately the reproductive activity of the oysters. The relationship between the index and the reproductive activity of the oysters was relatively close (Fig. 11). The highest peaks in the values of the index, recorded in December and April, indicated the gonads to be in active development or sexually ripe. The lowest values of the index, detected in March

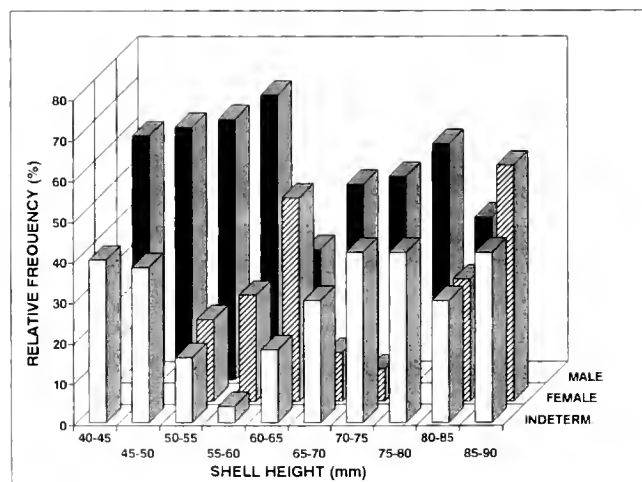


Figure 9. Size-related sex ratio in *P. sterna*.

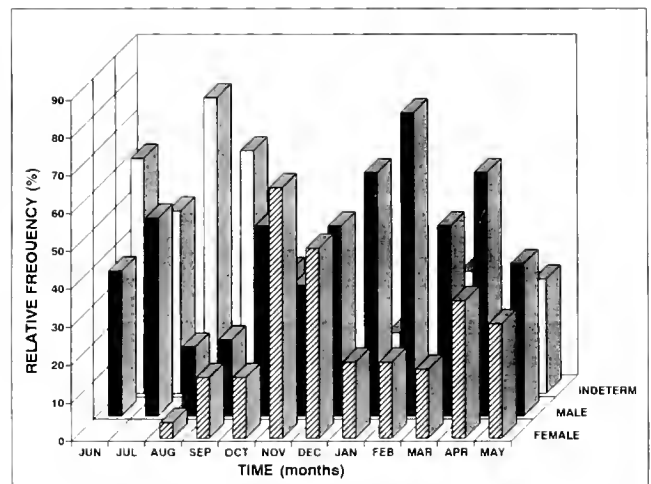


Figure 10. Temporal sex ratio in *P. sterna*.

and May, coincided with the two spawnings observed in the year. The GI and the water temperature had an inverse relationship.

DISCUSSION

As suggested by Giese and Pearse (1974), pearl oysters from temperate regions generally exhibit discrete and regular breeding seasons. Evidence found in this study indicated that *P. mazatlanica* and *P. sterna* followed a clear annual breeding cycle. Gametogenesis was found to be a continuous process throughout the annual cycle in both species. Changes observed in the reproductive activity of the oysters during the annual cycle were regulated mostly by seasonal changes in the water temperature. Earlier work done on pearl oyster reproduction from different parts of the world confirms Orton's rule: "if temperature conditions are constant or nearly so and the biological conditions do not vary much, animals will breed continuously" (Orton 1929 in Chellam 1987).

Tranter (1958b-d) observed a definite annual reproductive cycle for *Pinctada albina* and *Pinctada margaritifera* from the Torres Strait, Queensland. Sevilla (1969) also found a breeding cycle with continuous gametogenesis in *P. mazatlanica* from Bahía de La Paz, México. Rose et al. (1990) noted the same pattern of gametogenesis for *Pinctada maxima* from Eighty-Mile Beach, Western Australia, and García-Domínguez et al. (1996) made

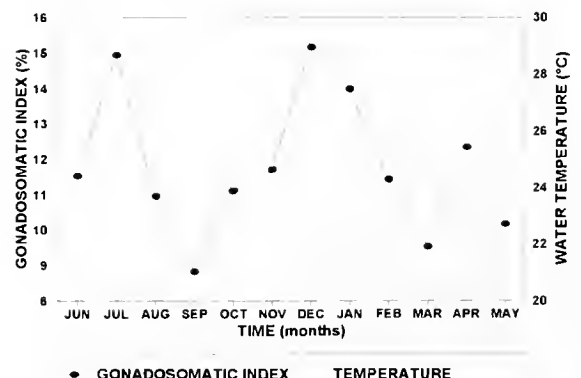


Figure 11. Relationship between the GI and the water temperature for *P. sterna*.

similar observations for wild *P. mazatlanica* from Isla Espíritu Santo, Baja California Sur, México.

However, some differences in the overall pattern of gametogenesis of pearl oysters can be detected. *P. albina* breeds annually and spawns once between April and May (Tranter 1958c), whereas *P. maxima* breeds annually, but spawns twice, from October to December and from February to April (Rose et al. 1990). *P. margaritifera* also has a bimodal spawning pattern, from March to August and from September to February (Tranter 1958d). A smaller species, *Pinctada fucata*, has two spawning peaks, during June to September and December to February, at Tuticorin Harbour (Chellam 1987). The tendency for a bimodal spawning pattern has been described for several marine invertebrates (Giese 1959, Giese and Pearse 1974).

During our study, *P. mazatlanica* bred annually and spawned once, from September to October, when the water temperature rose to 29–30°C. Histological evidence indicates a second short spawning could have occurred in June or July, triggered again by changes in water temperature. Unfortunately, the monthly sampling used for collecting the gonads did not allow us to detect this. *P. sterna* also bred annually, but spawned twice during the annual cycle. The maximum peak was from February to March, when the water temperature decreased to 24°C. The lesser peak was from April to May, when the water temperature dropped to 22.8°C. Once again, the histological evidence suggests the possibility of a third spawning, during June or July. We believe that *P. sterna* is potentially capable of spawning throughout the year, because mature gonads of both sexes were present almost all year. Rose et al. (1990) found mature oysters outside the main breeding period, suggesting that *P. maxima* is also capable of spawning all year.

The histological gonad analysis revealed that spawning in *P. mazatlanica* and *P. sterna* was not complete during the breeding season, and a large number of residual gametes were present after the spawning at the spent stage. We found phagocytic activity in oysters of both species that had recently spawned, indicating the presence of gonad regression. Tranter (1958c) and Rose et al. (1990) detected incomplete spawning and gonad regression in *P. albina* and *P. maxima*. However, regression in *P. sterna* was incomplete after the first massive spawning in February. We suggest the possibility of a second short breeding cycle in which animals avoid the spent stage and pass directly to gametogenesis after the first spawning. Similar observations were made by Sevilla (1969) for *P. mazatlanica*.

Pearl oysters seem to be functional protandrous hermaphrodites, with sexes separated by time. However, bisexual phases may occur in the same gonad, although they appear to be transitional and nonfunctional, as suggested by Rose et al. (1990) for *P. maxima*. Two cases of hermaphroditism were detected in *P. sterna*, both after 60-mm shell height. Similarly, García-Domínguez et al. (1990) found two hermaphrodite specimens in wild *P. mazatlanica*.

In this study, we managed young oysters instead of adults, especially in *P. mazatlanica*, a larger species. The total sex ratio was completely skewed to the male sex in both species, and therefore, they behaved as protandrous hermaphrodites. Particularly in *P. mazatlanica*, the female:male sex ratio of 0.12:1 and the mean sex-reversal size detected at 100 mm confirmed this. No females were observed under 100 mm. García-Domínguez et al. (1996) found a different female:male sex ratio of 1.33:1 in *P. mazatlanica* from Isla Espíritu Santo. However, although females outnumbered males, the study was carried out with larger individuals (adults),

ranging from 72 to 176-mm shell height. A sex ratio of 1:1 was observed for *P. maxima* from Eighty-Mile Beach, Western Australia, at 200-mm shell height (Rose et al. 1990).

For *P. sterna*, there was not enough evidence to conclude that the species were protandrous hermaphrodites, although the female:male sex ratio was 0.38:1. Females were present >50-mm shell height, but males kept appearing with high frequency up to 85 mm.

Apparently, all members of the genus *Pinctada* exhibit this capacity to change sex at a certain size, after male maturity has been reached. Previous descriptions of this phenomenon have been made by Sevilla (1969) for *P. mazatlanica*; Wada (1953a), Tranter (1958a), and Rose et al. (1990) for *P. maxima*; Wada (1953) and Ojima and Maeki (1955) for *Pinctada martensii*; Tranter (1958d) for *P. margaritifera*; and Tranter (1959) for *P. fucata*. However, change in sex can be reversible and may be brought about by stress (Cahn 1949, Tranter 1958a–d, Chellam 1987, Rose et al. 1990). The ability of sex reversal has been observed in other bivalves like the *Ostreidae*, *Teredinidae*, and *Pectinidae*, and as hypothesized by Tranter (1958b) for *P. albina*, it may be explained by a “weak hereditary sex-determining mechanism.”

Histological data demonstrated that male maturity was reached at 39 to 49-mm shell height for *P. mazatlanica*. This size range, corresponding to 8 months olds, was reached using organisms reared by extensive culture. *P. sterna* maturity was detected at 40- to 45-mm shell height, corresponding to 11 months olds. However, because *P. sterna* is a relatively small species, we believe that male maturity can be attained at a lower size, and the density inside the pearl cages could have somehow inhibited gonad maturity. Chellam (1978) found for *P. fucata*, a similar species in size, male maturity within 8 months and spawning at 9 months in Tuticorin Harbour. Tranter (1958a) also noticed *P. albina*, another small species, to be mature and spawn at 4 months. Male maturity occurs for *P. maxima* at 110–120 mm during the first year (Rose et al. 1990). Full maturity is not attained by *P. margaritifera* until the second year (Crossland 1957).

The GI was a useful quantitative method for estimating the reproductive activity of pearl oysters. Resting on the assumption that the ratio of body parts varies little with change in size of the animal (Giese and Pearse 1974), we were able to measure the relative reproductive condition of the oysters of different sizes and to compare changes in their gonads at different times. In species possessing little nutritive tissue in the gonads, like pearl oysters, an increase in GI was interpreted as a buildup of gametogenesis, with a decrease interpreted as spawning (Giese 1959). Because weight and volume values increase by approximately the cube of linear dimensions (Galtsoff 1931), care should be taken to equate dimensions when volumetric and linear measurements are used, as occurred in this study.

However, a limitation of the GI is that, unless accompanied by microscopic examination of the gonads, it indicates little as to what is occurring within the gonads. Therefore, if used as a single method, it could not be considered a reliable tool for studying the breeding cycle of pearl oysters and for understanding the seasonal changes occurring in their gonads. For pearl-culture programs, histology, coupled with gonad index measurements, is recommended for understanding the overall pattern of reproduction in pearl oysters.

Other aspects of the reproductive biology of pearl oysters *P. mazatlanica* and *P. sterna* are yet to be studied. To improve the techniques and strategies for spat collection, cultivation, growth, and especially, the production of high-quality cultured pearls, an-

nual and biannual trials on the breeding cycle of pearl oysters are recommended.

ACKNOWLEDGMENTS

We dedicate this work to the memory of Don Gastón Vivés, pioneer of pearl culture in the world. The study was conducted as part of an institutional program of the Centro de Investigaciones Biológicas del Noroeste (CIBNOR), México. It has also been funded by the International Foundation for Science (IFS of Sweden) since 1990, the Consejo Nacional de Ciencia y Tecnología

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THE EFFECT OF PENTACHLOROPHENOL ON PYRIDINE NUCLEOTIDE PRODUCTION IN OYSTER HEMOCYTES: NADPH AND IMMUNOMODULATION

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ABSTRACT Increased NADPH production coincides with the generation of reactive oxygen species (ROS) by immunostimulated hemocytes of the oyster, *Crassostrea virginica*. The effects of a putative environmental immunotoxicant on NADPH production and the subsequent effects on ROS generation are reported here. Oyster hemocytes were exposed in vitro to a range of sublethal concentrations of the biocide pentachlorophenol (PCP) for 20 h. The cells were then assayed for both NADPH and superoxide generation following immunostimulation. The results indicate that PCP partially inhibits the production of both NADPH and superoxide in a dose-dependent manner. Significant decreases in NADPH production were observed at 500 ppb, whereas significant decreases in superoxide generation were evident at 1,000 ppb. The decrease in NADPH production could represent a mechanism underlying the observed decrease in ROS production following PCP incubation.

KEY WORDS: Oyster hemocytes, pentachlorophenol, superoxide production, NADPH production, reactive oxygen species, immunotoxicity

INTRODUCTION

In the oyster *Crassostrea virginica* (Gmelin 1791), hemocytes are presumed to be important in the defense against pathogens (Anderson 1994). The production of reactive oxygen species (ROS) by hemocytes is one of the most prominent and intensely studied cell-mediated putative defense mechanisms in molluscs (Wishkovsky 1988, Adema et al. 1991, Anderson et al. 1995). There is a growing body of evidence that exposure to xenobiotics can modulate immune responses in aquatic organisms. The suppression of ROS production after in vitro exposure to toxicants has been used as a sensitive biomarker for immunomodulation (Tam and Hinsdill 1990). Although several chemical compounds have been shown to suppress ROS production in fish (Roszell and Anderson 1993, Anderson and Brubacher 1992, Warinner et al. 1988, Elasser et al. 1986) and oysters (Larson et al. 1989, Fisher et al. 1990, Roszell and Anderson 1992, Anderson et al. 1994), it is not clear if this is a manifestation of a generalized stress response or if toxicant-specific mechanisms are involved. The elucidation of specific mechanisms of toxicity would enhance the utility of immunomodulatory responses as biomonitoring tools.

ROS production in vertebrate phagocytes is well characterized (Robinson and Badwey 1992) and serves as the presumptive model for ROS production in bivalves (Anderson 1994). After immunostimulation, the membrane-associated enzyme NADPH oxidase catalyzes the transfer of a single electron from NADPH to molecular oxygen, producing the superoxide anion (O_2^-). Superoxide, while cytotoxic in itself, can be further metabolized to more highly toxic species. It can undergo dismutation to hydrogen peroxide, which, in turn, may be converted by Fenton chemistry into hydroxyl radicals (Halliwell and Gutteridge 1989). The enzyme myeloperoxidase, found in mammalian neutrophils as well as oyster hemocytes, catalyzes the production of hypohalous acids, such as HOCl, from H_2O_2 (Rosen and Klebanoff 1985). Several other interactions among the ROS are possible, generating a variety of oxygen species. Each of these reactive oxygen compounds has cytotoxic properties, inducing lipid peroxidation and enzyme inactivation.

Increased production of NADPH, chiefly by an up-regulation

of the pentose phosphate pathway, is essential to the production of O_2^- by NADPH oxidase. The purpose of this study was to measure the effects of a putative immunotoxicant on NADPH production in oyster hemocytes and to examine the relationship between NADPH and O_2^- production. The pesticide selected for this study, pentachlorophenol (PCP), is a potent general biocide and common aquatic pollutant (Ahlborg and Thunberg 1980). PCP is an uncoupler of oxidative phosphorylation; the resultant decrease in ATP production and subsequent effect on NADPH production were postulated to contribute to the observed immunotoxicity.

MATERIALS AND METHODS

Hemocyte Collection

Oysters, collected from the Wicomico River in St. Mary's County, MD, were maintained in a flow-through tank under ambient temperature (4–7°C) and salinity (10 ppt) conditions. The hemocytes were extracted as previously described (Anderson et al. 1995). Briefly, the oysters were notched and hemolymph was collected with a syringe from the adductor muscle sinus. The pooled hemolymph (nine oysters/pool) was plated onto glass Petri dishes and incubated at room temperature (22–23°C) for 15 min. Non-adherent cells were gently removed by rinsing with filtered ambient sea water (FA), and the adherent cells were incubated in FA at room temperature for an additional 2 h. After the incubation, the cells were collected by gentle aspiration and centrifuged at 300 g for 15 min. The FA was decanted, and the cell pellet was resuspended in Hanks Balanced Salt Solution (HBSS), made isotonic with NaCl, and augmented with 1 mg/mL glucose and 3% antibiotic/antimycotic solution (Sigma).

Pentachlorophenol Exposure

Hemocytes (10^6 cells/mL) were incubated with PCP ranging from 100 to 1,000 ppb (O_2^- studies) or 10 to 1,000 ppb (NADPH studies) for 20 h at room temperature. Stock solutions, made from water-soluble sodium PCP (Aldrich), ranged from 1.0 to 100 ppm such that an equal volume of stock was added to each treatment vial. Viability after incubation was assessed by use of the trypan

blue exclusion assay (equal volume of 0.4% trypan blue in HBSS and cells suspended in HBSS; incubation time = 5–10 min) and was based on four pools of cells.

Measurement of NADPH Production

NADPH production by chemically stimulated oyster hemocytes was estimated with the CellTiter 96 AQ kit (Promega), which is a colorimetric assay based on the production of reduced pyridine nucleotides. After a 20-h incubation with PCP, each pool of cells was centrifuged, decanted, resuspended in fresh HBSS (iso-osmotic, augmented with 1 mg/mL glucose), and divided equally into 96-well plates (200,000 cells/well, six wells/treatment per pool.) Superoxide dismutase (300 U/mL, final concentration) was added to each well to prevent any spurious interaction between O_2^- and the assay reagents. The NADPH oxidase stimulator phorbol 12-myristate 13-acetate (PMA, Sigma) was added to half of the wells of each treatment group to give a final concentration of 0.001 mM. Cells were incubated for 20 min at room temperature, and then the reagent mixture was added. Incubation continued for an additional 60 min, at which time the color change was read on a Bio-Rad model 2550 EIA Reader at 492 nm. Three separate pools of hemolymph were analyzed to permit statistical evaluation.

The use of this assay to evaluate NADPH production with immunostimulation represents a novel application because the Promega CellTiter 96 AQ kit is traditionally used to characterize viability or cell proliferation. The kit consists of two reagents: the sulfated, water-soluble tetrazolium, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS), and phenazine methosulfate (PMS), which acts as an electron transfer intermediary. Reduced pyridine nucleotides complex with PMS, allowing for a two-electron transfer to occur, forming *N*-methyl dihydrophenazine. This compound is capable of a single-electron transfer to the tetrazolium, resulting in the formation of formazyl and phenazyl radicals. The transfer of the second electron results in the production of a blue formazan and the regeneration of PMS (Dunigan et al. 1995). This reaction can be monitored colorimetrically at 490 nm. Phagocytic cells produced NADPH on appropriate immunostimulation; this was detected by absorbance readings that were elevated over the concomitant baseline production of reduced pyridine nucleotides (NADPH and NADH) by the cells.

Measurement of Superoxide Production

The effect of PCP on O_2^- production was tested by the use of lucigenin-augmented chemiluminescence (CL). After incubation with PCP, lucigenin (*bis-N*-methylacridinium nitrate, Sigma) was added to each incubation vial (25 μ M, final concentration), and the vials were loaded into a Packard Tri-Carb 1900 CA liquid scintillation analyzer, adapted for single-photon counting. After baseline measurements were obtained, zymosan (Sigma), at a concentration of 4 mg/mL, was added, and CL was monitored for approximately 2 h. Cell-free experiments with xanthine/xanthine oxidase as the O_2^- -generating system demonstrated that the presence of PCP does not interfere with the detection of O_2^- ($p = 0.4069$, data not shown). The assay was repeated four times with four pools of hemolymph.

Statistics

All data were analyzed with the Prism™ (GraphPad) statistics package. Viability data were analyzed by the use of analysis of

variance (ANOVA). For both NADPH and O_2^- production data, repeated-measures ANOVA was used, because variation between pools was expected to be significant. Both baseline NADPH production (unstimulated cells) and net NADPH production (estimated by subtracting the absorbance of unstimulated cells from the absorbance of stimulated cells) were evaluated. CL data were analyzed in terms of both area under the curve and peak CL minus unstimulated baseline. Two post-hoc tests were used when the ANOVA showed significant ($p < 0.05$) variation between study groups: Dunnett's multiple comparison test to identify differences between control and treatment means, and trend analysis to test for a linear relationship among the means.

RESULTS

Exposure to $\leq 1,000$ ppb PCP did not significantly affect hemocyte viability ($p = 0.3886$, data not shown). The experimental design incorporated the use of repeated-measures ANOVA to analyze the baseline NADPH, net NADPH, and O_2^- data; the pairing was statistically significant at $p < 0.001$, $p < 0.005$, and $p < 0.0001$, respectively, indicating that the matching was effective and that the use of this method was appropriate. Exposure to PCP resulted in no significant differences in the baseline production of NADPH ($p = 0.8451$, data not shown); however, significant inhibition of stimulated net NADPH production ($p = 0.0137$) was evident at both 500 and 1,000 ppb (Fig. 1). The evaluation of the integrated area under the curve for O_2^- generation indicated significant decreases at 1,000 ppb ($p = 0.0178$; Fig. 2). The analysis of peak minus baseline data gave similar results ($p = 0.0166$, data not shown). In all instances, the relationships between the control and treatment means were linear ($p < 0.005$), indicating significant dose-response relationships.

DISCUSSION

In mammals, the enzyme NADPH oxidase facilitates the transfer of an electron from NADPH to molecular oxygen to produce O_2^- . Although direct evidence of the presence of this enzyme in oyster hemocytes is lacking, there is circumstantial evidence of its existence. Hemocytes share several important characteristics with mammalian blood cells, including phagocytic capacity, the production of ROS, and the generation of effector molecules such as lysozyme that may function in concert with ROS. Like their mam-

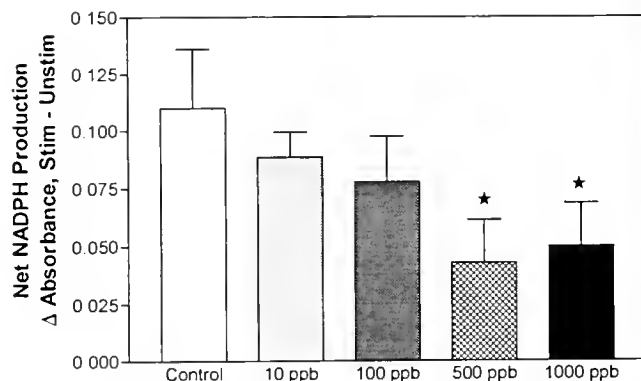


Figure 1. NADPH production by oyster hemocytes after 20-h in vitro exposure to PCP (200,000 cells/well, $n = 3$). Stars indicate means that differ significantly from control (Dunnett's post-hoc test, $p < 0.05$). Stim, stimulated; Unstim, unstimulated.

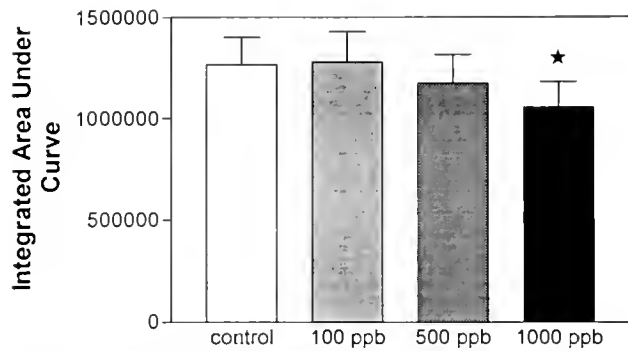


Figure 2. Lucigenin-augmented CL after 20-h in vitro exposure to PCP (10^6 cells/mL, integrated area under the curve, $n = 4$). Star indicates mean that differs significantly from control (Dunnett's post-hoc test, $p < 0.05$).

malian counterparts, hemocytes respond to immunostimulation by zymosan or PMA by producing ROS, although their response is lower in magnitude. Inhibitors of NADPH oxidase activity have been shown to decrease CL in the hemocytes of molluscs such as *Lymnaea stagnalis* (Adema et al. 1993) and *Mytilus edulis* (Noel et al. 1993). Therefore, it is not unreasonable to assume that an enzyme homologous to NADPH oxidase may be active in oyster hemocytes. Recent evidence indicates that ROS production by oyster hemocytes may not be elicited, or may even be suppressed, by exposure to certain viable, potentially pathogenic bacteria (Bramble and Anderson, in press) or the protozoan parasite *Perkinsus marinus* (La Peyre et al. 1995), suggesting that other factors may also contribute to cell-mediated defense. However, by analogy to the better characterized mammalian systems, ROS production may be considered a measure of the defensive capacity of oyster hemocytes and the inhibition of this response could impose a limitation on disease resistance.

In phagocytic cells, the NADPH used to fuel the respiratory burst is generated by the pentose phosphate pathway, which uses glucose-6-phosphate (G6P) supplied by glycolysis or glycogen metabolism. ATP is required in several steps in the production of G6P by glycolysis, as well as in the activation of the enzymes phosphorylase kinase and glycogen phosphorylase—both of which

are necessary for the cleavage of G1P from glycogen. ATP may also be necessary for the assemblage of NADPH oxidase. PCP, an uncoupler of oxidative phosphorylation, is similar to dinitrophenol (Cantelmo et al. 1978) in that it acts as a proton shuttle across mitochondrial membranes, depleting the proton gradient required for ATP production. Because ATP is required for NADPH production, PCP has the potential to limit the availability of NADPH for ROS production.

The data presented here indicate that in vitro exposure to PCP inhibits NADPH production by immunostimulated hemocytes. Although both O_2^- production and NADPH production by oyster hemocytes are decreased after exposure to PCP, significant decreases in NADPH appear at lower concentrations of PCP than does decreased O_2^- production. These results suggest that the decreased NADPH production is the proximate cause of the decreased ROS production. The fact that NADPH appears to be slightly more sensitive to PCP implies that the coupling between NADPH production and superoxide generation is not tightly linked. This is not unexpected, because NADPH is an important cofactor in numerous other cellular functions unrelated to the production of ROS.

In summary, oyster hemocytes exposed in vitro to PCP exhibited decreased CL in response to phagocytic stimulation. They also demonstrated significantly decreased NADPH production in response to chemical stimulation with PMA. Because NADPH is a required cofactor in the production of O_2^- , it appears that the immunosuppressive action of PCP results from the reduced NADPH production. Other possible explanations for decreased O_2^- production include direct interference with NADPH oxidase assembly or altered cellular redox status (decreased NADPH: NADP⁺), leading to lipid peroxidation and enzyme inactivation. Because O_2^- production may be an element of microbicidal defense in oyster hemocytes, the inhibition of this pathway by exposure to environmental contaminants could have dire consequences in terms of resistance to infectious diseases (Anderson et al. 1996, Chu and Hale 1994). However, a concrete relationship between decreased O_2^- production and increased susceptibility to disease cannot be established until the role of O_2^- production in disease resistance in oysters is completely characterized.

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OYSTER SHELL DISARTICULATION IN THREE CHESAPEAKE BAY TRIBUTARIES

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ABSTRACT We examined the effects of site location, season of deployment, substrate, size class, and salinity and temperature regimen on the time-since-death (TSD) required for the disarticulation of shells of *Crassostrea virginica* (Gmelin, 1791) at sites located in three Chesapeake Bay tributaries. The mean TSD required for disarticulation was greatest at Chestertown (815.5 days), intermediate at Oxford (718.9 days), and least at Deal Island (630.0 days), which corresponded to progressively increasing mean salinities. The mean TSD for all sites combined was 739.4 days, ranging from 21 to 1,427 days. Within sites, oyster shell size class (i.e., market-sized >76 mm, small <76 mm) had significant intrasite effects on mean TSD at Chestertown and Oxford, although among sites, a significant interaction existed between size class and sites. Overall, shell length had a strong positive correlation with TSD and accounted for 10.0% of the variability in TSD. Mean annual salinity had a strong negative correlation with TSD, accounting for 18.1% of the variability in mean TSD. The season of deployment of oyster shells had a significant effect on the mean TSD at Chestertown and Oxford, although not in a consistent manner from one site to another. However, overall, the cumulative percentage disarticulation was greatest in summer (47.6%) and least in winter (6.3%). Among sites, the substrate on which the trays were deployed (i.e., reef or sediment) did not significantly affect mean TSD.

KEY WORDS: *Crassostrea virginica*, oyster mortality, box counts, shell disarticulation

INTRODUCTION

The ability to accurately determine the interval since the death of bivalves, referred to as time-since-death (TSD), is important in evaluating mortality in such populations. After a bivalve dies and its tissues decompose, in the absence of disturbance or degradation, both valves of its shell remain intact because of the attachment of each valve to the proteinaceous hinge ligament. Such articulated shells form what is referred to as a box, until disarticulation eventually occurs. Such box records for the eastern oyster *Crassostrea virginica* have been used extensively in assessing oyster mortality levels (Christmas and Jordan 1990), in estimating oyster abundance and mortality (Homer et al. 1993), and in annual oyster surveys (Krantz 1992, Krantz 1993).

However, it is generally accepted that there is a wide range of variability in the accuracy of estimates of TSD obtained by the so-called box count method. J. D. Andrews (Pers. comm., Virginia Institute of Marine Science [VIMS]) concluded that box counts were not reliable indicators of either TSD or mortality rate, primarily because of the effects of salinity, bottom type, season, and competition among fouling organisms on disarticulation rates. Mackin (1961) concluded that without an understanding of the rate of disarticulation in a particular area, mortality rates obtained from box counts were meaningless. Mackin further asserted that the only legitimate use of box counts was in the assessment of recent "cataclysmic" mortality, because rates of disarticulation were so poorly understood.

Few long-term studies have addressed the question of how long it takes for oyster shells to disarticulate. Gunter and Dawson (1957) did not observe any disarticulation of oyster boxes in a

study conducted in Port Arkansas, TX, although their study lasted only 73 days. In a study conducted in Barataria Bay, LA, Mackin (1949) found that 20–38% of the oyster boxes he studied disarticulated after 6 mo.

Although little information is available on the validity of the box count method, it has remained in use in recent years because it is a convenient, cost-effective method of assessing oyster mortality. The objective of this study was to evaluate the effects of site location, season of mortality, size, substrate, and salinity and temperature regimen on TSD in order to evaluate the effectiveness of the box count method.

METHODS

Study Site

This study was conducted at three mesohaline riverine sites situated on the Eastern Shore of Maryland (Fig. 1), within the Chesapeake Bay watershed. The study sites, Chestertown, Oxford, and Deal Island, were located, respectively, in the Chester River (39°06'39"N, 76°08'13"W), Tred Avon River (38°40'46"N, 76°10'27"W), and Manokin River (38°10'08"N, 75°56'48"W). Water quality is generally good in the Tred Avon River, with minimal pollution, whereas water quality is fair in the Chester and Manokin Rivers, characterized by continued low-level degradation (Maryland Department of the Environment 1993). Natural oyster bars historically were present in each of the study areas (Merritt 1977). The watershed size of each of these tributaries, in Maryland, is as follows: Chester River, 1,816 km²; Tred Avon River, 122 km²; and the Manokin River, 88 km².

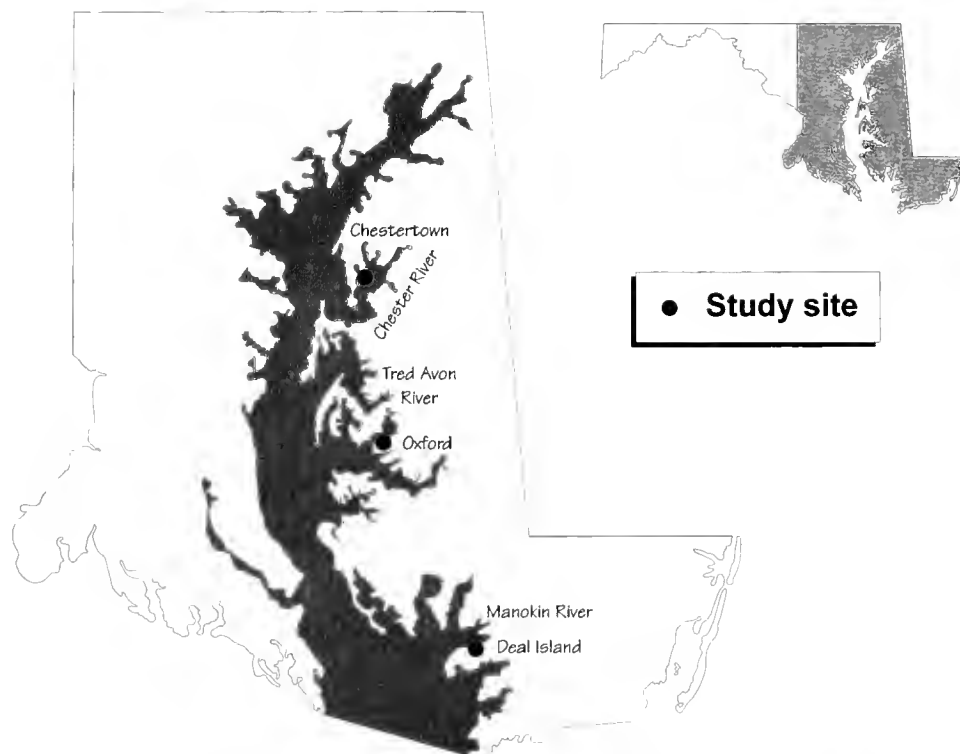


Figure 1. The Chesapeake Bay in Maryland, showing the Chestertown, Oxford, and Deal Island study sites.

Deployment of Oyster Trays

From July 30, 1991, to May 4, 1992, oysters were sacrificed and deployed seasonally (summer, fall, winter, spring) in trays that were attached to pier pilings and rested on the bottom. The dates of deployment were as follows: July 30, 1991; October 28, 1991; January 30, 1992; and May 4, 1992. Live oysters were collected, as needed, from either the Choptank or Potomac Rivers. Initially, a sample of 60 market-sized (>76 mm) and 60 small oysters (<76 mm) were sacrificed by immersion in a bath of concentrated KCl. For subsequent deployments, oysters were sacrificed by the injection of 10–14 mL of saturated KCl solution through a 0.6-cm hole drilled through the right valve of the oyster.

The oysters were then transferred to 0.60-m-wide \times 0.60-m-long \times 0.15-m-deep trays. Two trays were deployed at each site during the initial deployment in the summer of 1991: a reef tray and a sediment tray. The reef trays were raised from the bottom by three sections of wood that were arranged into an H-shaped frame attached to the base of the tray. The frame dimensions were 2 cm wide \times 51 cm long \times 10 cm deep. The sediment trays, however, were designed to rest directly on the substrate. Initially, the reef trays were uncovered while the sediment trays were covered with lids made of 1.25-cm mesh hardware cloth. For subsequent deployments (i.e., fall 1991, winter 1992, and spring 1992), only reef trays were used. Because the original trays began to disintegrate within several months, they were replaced with polypropylene trays of a similar design. Replacement reef trays were also fitted with lids, because wave action and storm events resulted in the periodic loss of oysters from uncovered trays.

Sampling Procedure

Trays were monitored biweekly for the first year of the study and thereafter at monthly intervals. During each sampling period, the trays were retrieved manually and a box count was made. Any occurrence of disarticulation was recorded, and the TSD was calculated in days. The shells of all disarticulated oysters were removed and measured, and it was noted if both valves were recovered and whether the right valve retained the identifying hole that was drilled, initially, to allow the injection of KCl. Disarticulation was recorded only when such a drilled right valve was found. Sampling was continued until all of the oysters deployed had disarticulated. Salinity and water temperature were measured with a YSI Model 33 SCT meter during each sampling event.

Statistical Analysis

Analysis of variance (ANOVA) (PROC GLM; SAS Institute 1985) was used to examine differences among and within sites in relation to TSD. A $3 \times 4 \times 2$ factorial design was used to evaluate variation in TSD, with site, season of deployment, and size class as factors. A $3 \times 2 \times 2$ factorial design was used to evaluate variation in TSD with site, substrate (reef and sediment), and size class as factors. At each site, a 4×2 factorial design was also used to evaluate intrasite variation in TSD, with season of deployment and size class as factors. A $2 \times 2 \times 4$ factorial design was used to make multiple comparisons of TSD between sites, using size class, site, and season of deployment as factors.

We used Spearman rank correlation analysis to examine the association between shell length and TSD at each site. Least squares regression analyses were used to examine the relationships between combined shell lengths at all sites and TSD and to exam-

TABLE 1.

Mean, standard deviation (SD), and range of the TSD required for the disarticulation of oyster shells at Chestertown, Oxford, and Deal Island with all treatments and seasonal deployments combined (n = 819).

Site	Mean TSD	SD	Minimum	Maximum	n
Chestertown	815.5	216.3	204	1,427	364
Oxford	718.9	198.5	21	1,238	265
Deal Island	630.0	269.6	21	1,366	190

ine the relationships between mean salinity and mean TSD for all treatments.

RESULTS

Overall Comparison of Oyster Shell Disarticulation

Although a total of 1,800 articulated oysters were deployed over the course of the study, only 819 (45.5%) of the disarticulated oysters were recovered. Of the 981 oysters that were not recovered, the reasons for the losses were as follows: failure of KCl injection to induce mortality, 9.2%; vandalism of trays, 11.3%; and deterioration of trays, 79.5%.

With all treatments and sites combined, the overall mean TSD required for the disarticulation of oyster shells was 739.4 days. Table 1 shows the mean, standard deviation, and range for all sites with all treatments combined. The mean TSD was least at Deal Island, intermediate at Oxford, and greatest at Chestertown. For all sites combined, the TSD ranged from 21 to 1,427 days. Table 2 shows the mean, standard deviation, and range of the TSD for all treatments at each site.

Reef Treatments

With a three-way factorial ANOVA among reef sites (Table 3), size class, site, and season of deployment each had a significant

effect on TSD. However, there was significant interaction between site and season such that the main effects of these factors should be interpreted cautiously. When sites were examined individually, using a two-way factorial ANOVA, with season of deployment and size class as factors (Table 4), both season of deployment and size class had significant effects on mean TSD at Chestertown and Oxford, with no significant interaction. At Deal Island, however, there was significant interaction between season of deployment and size class. Although intrasite seasonal differences were significant at Chestertown and Oxford, the effect of the particular season of deployment on disarticulation varied inconsistently from one site to another. Figure 2 shows the mean TSD for oysters deployed during various seasons at each site. The cumulative percent disarticulation that occurred during each season, for all sites and treatments combined, was: summer, 47.6%; fall, 28.8%; winter, 6.3%; and spring, 17.3%.

Paired multiple comparisons of the three sites, using a three-way ANOVA, with site, size class, and season of deployment as factors, showed that site had a significant effect on TSD between Deal Island and Chestertown ($F = 9.29$, $p = 0.003$), as well as between Oxford and Chestertown ($F = 5.16$, $p = 0.024$), but not between Deal Island and Oxford ($F = 0.59$, $p = 0.4431$). Size class and season of deployment had a significant effect on TSD in all comparisons between sites.

At all sites, less than 25% of the oyster shells in each treatment had disarticulated after a period of 1 y, regardless of the season in which they were deployed (Fig. 3). At both Chestertown and Deal Island, disarticulation rates varied considerably between seasons of deployment. However, at Oxford, the differences among seasonal deployments were less pronounced. For all sites, the highest rates of disarticulation were observed for the summer 1991 and winter 1991 deployments.

Reef Versus Sediment Treatments

A three-way factorial ANOVA of the reef and sediment treatments, with substrate, size class, and site as factors, showed that

TABLE 2.

Mean, SD, and range of the TSD required for the disarticulation of oyster shells (market sized and small combined) deployed in various seasons at three sites: Chestertown, Oxford, and Deal Island (n = 819).

Season Deployed			Mean TSD	SD	Minimum	Maximum	n
Chestertown							
Summer	1991	(sediment)	964.6	210.5	483	1,427	94
Summer	1991	(reef)	784.7	152.0	483	1,394	42
Fall	1991	(reef)	860.3	195.7	392	1,334	92
Winter	1991	(reef)	657.3	138.8	362	998	74
Spring	1992	(reef)	732.4	199.7	204	1,241	62
Oxford							
Summer	1991	(sediment)	716.2	219.1	21	1,152	92
Summer	1991	(reef)	631.7	164.1	392	799	9
Fall	1991	(reef)	744.4	182.5	231	969	27
Winter	1992	(reef)	703.6	167.3	364	1,238	98
Spring	1992	(reef)	790.9	214.6	455	1,177	39
Deal Island							
Summer	1991	(sediment)	552.6	223.5	21	1,366	72
Summer	1991	(reef)	770.7	162.9	378	1,185	22
Fall	1991	(reef)	740.1	252.2	246	1,093	14
Winter	1992	(reef)	528.3	289.3	94	1,213	40
Spring	1992	(reef)	753.7	290.3	127	1,241	42

TABLE 3.

Results of General Linear Models analysis of the TSD (days) required for disarticulation of oyster shells deployed in reef treatments testing the effects of site, size class, and season ($n = 561$).

Factor	Factor Levels	df	F Value	p Value
Site	Chestertown Oxford Deal Island	2	5.42	0.0214
Size Class	Market sized Small	1	28.53	0.0001
Season	Summer Fall Winter Spring	3	15.96	0.0001
Interaction	Site \times Size Class Site \times Season Season \times Size Class	2 6 3	2.75 3.81 2.41	0.0648 0.0010 0.0660

substrate did not have a significant effect on TSD, although the site and substrate interaction was significant. There was interaction between site and size class, such that the main effects of these factors should be interpreted cautiously (Table 5).

Shell Size Class and Length

A two-way ANOVA, with site and size class as factors ($F = 38.08$, $p = 0.0001$) showed that, among sites, the overall mean TSD of small oyster shells (691.2 days) was significantly less than that for market-sized oyster shells (791.5 days). The mean TSD of small and market-sized oyster shells was significantly different at all sites: Chestertown ($F = 7.01$, $p = 0.0086$), Oxford ($F = 6.02$, $p = 0.0152$), and Deal Island ($F = 19.44$, $p = 0.0001$). No significant interaction was observed between season and shell size class at any of the sites. Figure 4 shows a comparison of the mean TSD of both size classes at each site for each reef treatment.

Oyster shell length had a significant, positive correlation with TSD at all sites (Fig. 5). The correlation was strongest at Deal Island ($r = 0.485$, $p = 0.0001$), intermediate at Chestertown ($r = 0.323$, $p = 0.0001$), and weakest at Oxford ($r = 0.229$, $p = 0.0032$). Figure 6 shows the relationship of shell length and TSD for all sites combined. A least squares linear regression analysis showed that shell length had a positive relationship with TSD ($r^2 = 0.100$, $p = 0.0001$), accounting for 10.0% of the variability in TSD.

Temperature and Salinity

The minimum and maximum water temperatures measured during this study were 0.1 and 32.5°C, respectively. Mean water temperature was 15.2°C at Chestertown, 15.5°C at Oxford, and 16.2°C at Deal Island. Overall, water temperature varied little among the sites (Fig. 7). A least squares linear regression analysis showed no significant relationship between mean TSD and mean water temperature, when averaged over the duration of the study at each site ($r^2 = 0.071$, $p = 0.173$). The seasonal differences in cumulative percent disarticulation corresponded to variations in water temperature, with disarticulation more frequent during

TABLE 4.

Results of General Linear Models analysis of the TSD (days) required for disarticulation of oyster shells deployed in reef treatments during the summer and fall of 1991 and winter and spring of 1992, testing the effects of size class and season within each site.

Factor*	Factor Levels	df	F Value	p Value
Season	Summer, Fall, Winter, Spring			
Chestertown		3	20.99	0.0001
Oxford		3	4.46	0.0131
Deal Island		3	4.48	0.0052
Size Class	Market sized, Small			
Chestertown		1	15.83	0.0001
Oxford		1	4.03	0.0465
Deal Island		1	5.24	0.0239
Interaction	Season \times Size			
Chestertown		3	1.03	0.3786
Oxford		3	1.96	0.1437
Deal Island		3	2.92	0.0373

* Chestertown, $n = 270$; Oxford, $n = 173$; Deal Island, $n = 118$.

warmer months than during colder months. Over the duration of the study, with all treatments and sites combined, 53.9% of the disarticulation occurred during the period from June 1 to September 30. Figure 8 shows the cumulative percent disarticulation that occurred during each month, with all treatments and sites combined. Overall, disarticulation occurred most frequently in August (15.3%), September (14.5%), and July (12.6%).

The minimum and maximum salinities measured during this study were 2.8 and 19.2 ppt, respectively. Mean salinities varied considerably among sites: 9.6 ppt at Chestertown, 12.6 ppt at Oxford, and 14.5 ppt at Deal Island (Fig. 7). A least squares linear regression analysis of mean TSD on mean salinity (Fig. 9) showed that mean salinity, averaged over the duration of the study, accounted for 18.1% of the variability in mean TSD ($r^2 = 0.181$, $p = 0.064$). Overall, less time was necessary for the disarticulation of oyster shells at sites with higher mean salinities. However, Figure 7 shows that on an annual basis, at each site, periods with higher salinities corresponded to periods with lower temperatures.

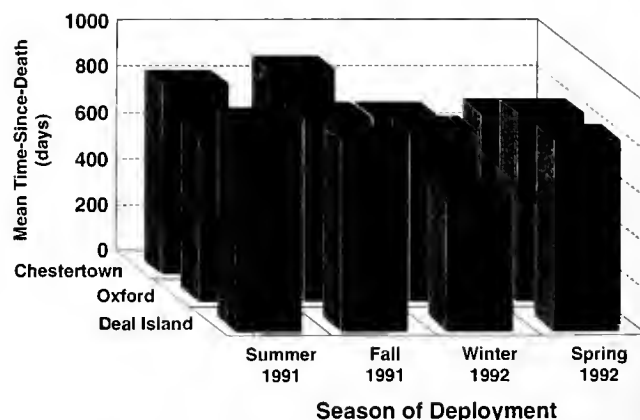
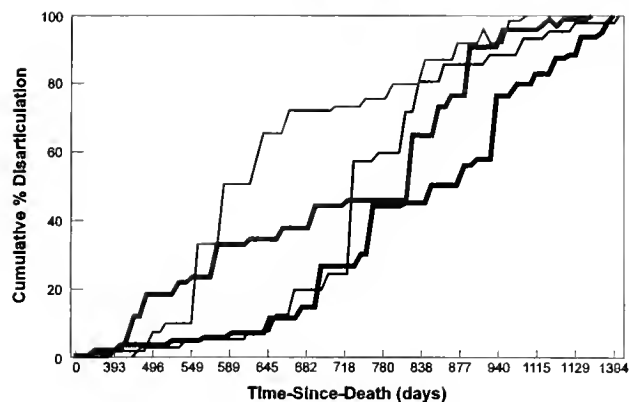
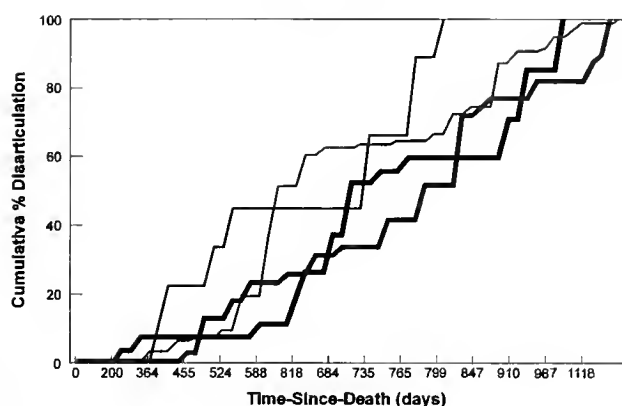
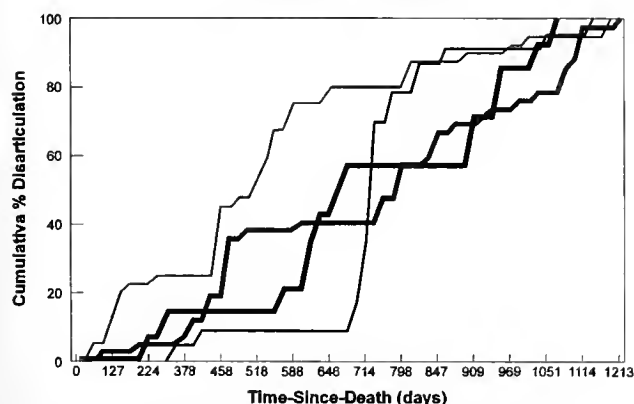


Figure 2. Comparison of mean TSD required for the disarticulation of oyster shells deployed during different seasons in reef treatments at Chestertown, Oxford, and Deal Island.

CHESTERTOWN**OXFORD****DEAL ISLAND**

SUMMER 91 FALL 91 WINTER 92 SPRING 92

Figure 3. Cumulative percent disarticulation of oyster shells deployed during different seasons in reef treatments at Chestertown, Oxford, and Deal Island.

DISCUSSION

In this study, the time required for the disarticulation of oyster shells varied both within and among different geographic regions. Mean TSD was longer than 2 y at all sites, which is considerably longer than the 1 y generally assumed in most assessments of oyster mortality, based on box counts. In all treatments at all sites,

less than 25% of the oyster shells had disarticulated after the first year of deployment. On the basis of this study, it appears that the interval from the occurrence of oyster mortality until the disarticulation of oyster shells can easily overlap several field sampling periods. Thus, oyster boxes, which are generally interpreted as representing mortality from only the preceding year, may in fact represent mortality from several years previous—as many as three or four—and the misinterpretation of such data may result in an overestimation of natural mortality.

Although the composition and function of the oyster hinge ligament are well known, little is known about the rates at which chemical decomposition and physical erosion affect hinge ligament dissolution. The ligament is composed primarily of organic material (conchiolin) and calcium carbonate and forms a nonliving, resilient material that articulates and seals the anterior portion of both valves of the oyster shell (Carriger 1996). The central portion of the ligament, the resilium, ranges from 30 to 67% calcium carbonate by weight and is reinforced with argonite fibers. The chemical composition of the lateral ligaments that flank the resilium on either side, by weight, is: calcium carbonate, 5.3–8.5%; protein, 33.9%; and carbohydrate, 0.1% (Galtsoff 1964, Kahler et al. 1976). Although little information is available on the rates of decomposition of the various structural components of the hinge ligament, the lamellar ligaments (i.e., tensilia) are most resistant to degradation (Chris Dungan pers. comm., Maryland Department of Natural Resources).

It appears that mean salinity, or a factor correlated with salinity, is one of the primary factors affecting the rate of shell disarticulation, accounting for 18.1% of the variability in TSD. One factor that covaries with salinity is the diversity of aquatic species in an estuary. There is a gradual decrease in species richness as oceanic salinities decrease, with species richness diminishing rapidly below 10 ppt, becoming minimal in the salinity range from 5 to 8 ppt (Remane and Schlieper 1975, Boaden 1986, Knox 1986), and increasing rapidly again in salinities less than 5 ppt.

Various species of proteolytic bacteria, distributions of which can be salinity and temperature limited, have been identified in aquatic environments. Their diversity would be expected, hypothetically, to follow the aforementioned salinity-related distribu-

TABLE 5.

Results of General Linear Models analysis of the TSD (days) required for disarticulation of oyster shells deployed in both reef and sediment treatments during the summer of 1991, testing the effects of shell size, site, and substrate ($n = 331$).

Factor	Factor Level	df	F Value	p Value
Site	Chestertown Oxford Deal Island	2	30.09	0.0001
Size Class	Market sized Small	1	7.81	0.0055
Substrate	Sediment Reef	1	0.37	0.5414
Interaction	Site \times Size Class	2	4.74	0.0094
	Site \times Season	2	19.08	0.0001
	Season \times Size Class	1	2.18	0.1407

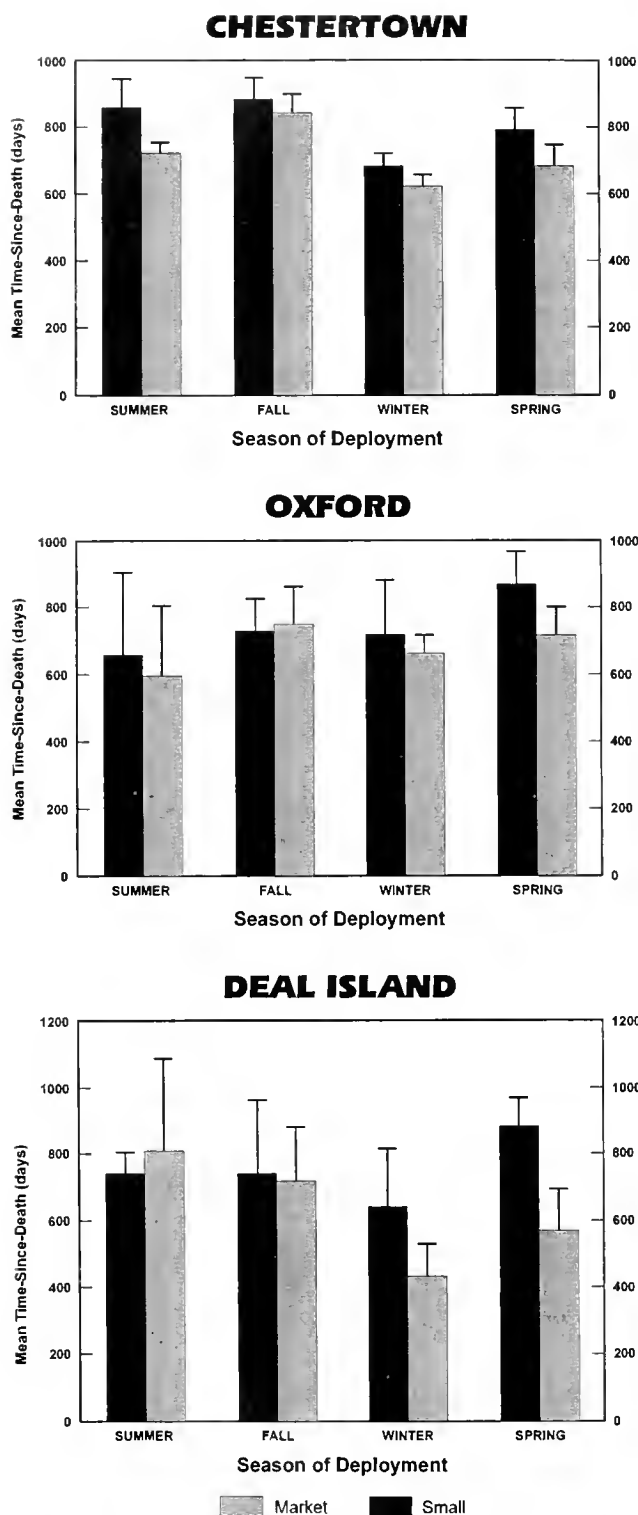


Figure 4. Comparison of the mean TSD required for the disarticulation of market-sized and small oyster shells deployed during different seasons at Chestertown, Oxford, and Deal Island (error bars represent 95% confidence interval).

served in this study. Sjogren (1982) observed that temperature changes significantly affected rates of proteolysis, with increased temperatures resulting in increased proteolytic activity. This could account for the observed seasonal trends in cumulative percent occurrence of disarticulation.

Similarly, the observed differences in the TSD of market-sized and small oyster shells could be explained by the variability in the time required for the bacterially-mediated degradation of the hinge ligament, as a function of the size of the hinge ligament. Dungan et al. (1989) isolated several strains of proteolytic cytophaga-like gliding bacteria (CLB) associated with the hinge ligaments of cultured juvenile Pacific oysters, *Crassostrea gigas*. In vitro, the CLB

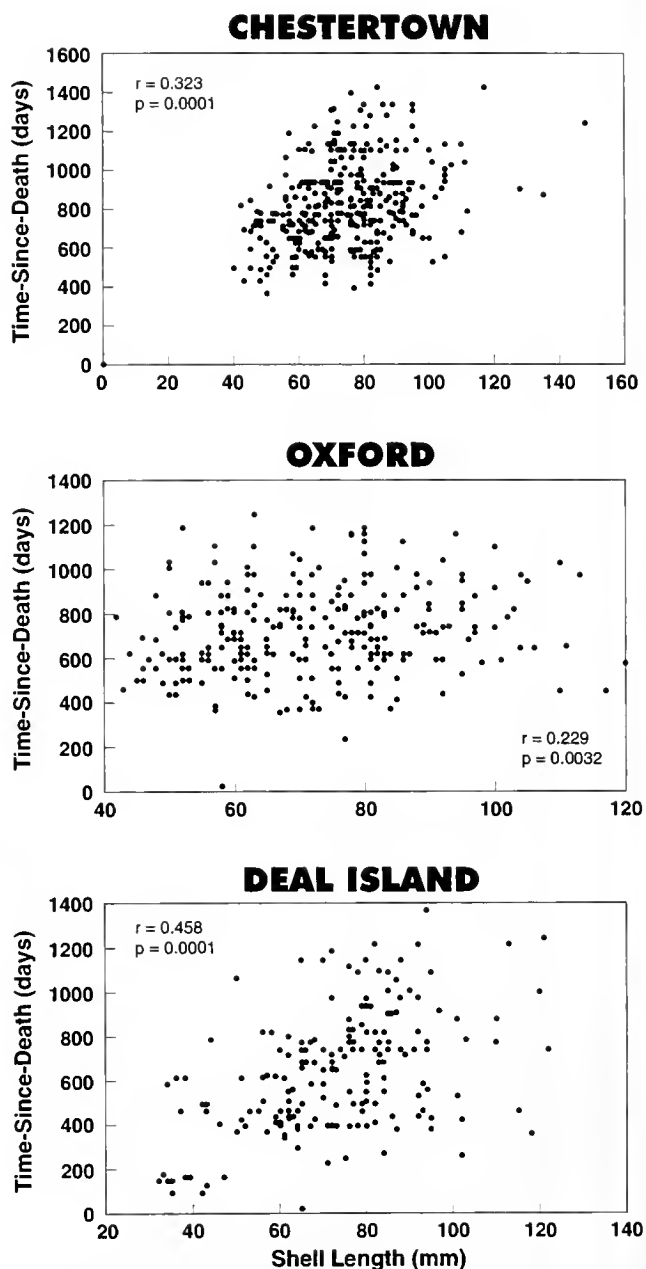


Figure 5. Relation of shell length (mm) and TSD required for disarticulation (days), and corresponding Spearman correlation values, for oyster shells deployed at Chestertown, Oxford, and Deal Island.

tional pattern. If the diversity of such proteolytic bacteria and other saprophytic taxa is greater in higher salinity areas, this could account for the relative rapidity of disarticulation in higher salinity waters in comparison with lower salinity waters, which was ob-

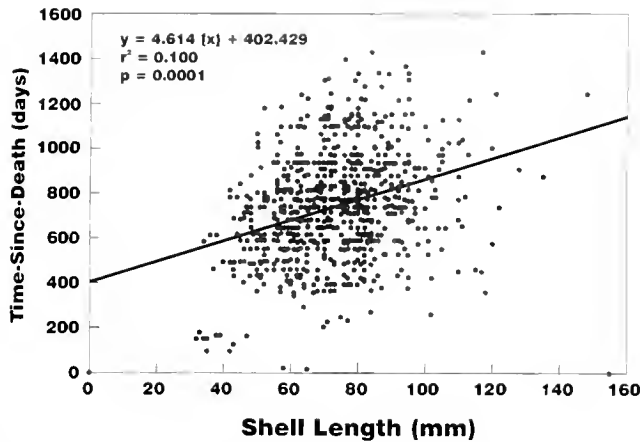


Figure 6. Relationship of shell size (mm) and TSD required for disarticulation (days) of oyster shells, with all sites and treatment combined.

strains survived with only hinge ligament resilium as the growth matrix. The initial degradation of the hinge ligament was followed by its liquefaction. If the primary biotic cause of oyster shell disarticulation is bacterially-mediated, then it would be expected that the more elaborate resilium of market-sized oysters would take longer to degrade than the less expensive resilium of smaller oysters. Such degradation of the hinge ligament would be necessary for disarticulation to occur in the absence of some physical disturbance. The differences observed in this study in the rates of disarticulation between market-sized and small oysters have been suggested by other investigators. Krantz (1992, 1995) noted the positive relationship between shell size and both ligament size and shell strength as well as the differential effects of the foraging behavior of predators on oysters of different sizes.

Limited efforts have been made to model bivalve mortality based on mortality data obtained from box counts. Merrill and Posgay (1964) found that the average time required for the disarticulation of sea scallops, *Placopecten magellanicus*, ranged from 26 to 36 wk. Furthermore, they were able to calculate an instantaneous natural mortality rate for this species, in the northeast part of Georges Bank. The model was based on the modal difference between the length-frequency distribution of the shells of live and dead scallops. However, because of our experimental design, it would be extremely difficult to develop similar models for natural mortality in our study areas. Primarily, this is because the length-frequency distribution of the oysters deployed was not representative of the resident oyster populations: in fact, none of the oysters were collected from the areas in which they were deployed and the only difference in length frequencies resulted from the selection of oysters as either small or market-sized.

On the basis of the results of this study, it appears that, in order to use oyster box counts to make inferences about natural mortality rates and TSD of oyster shells, additional studies incorporating more sites and using representative size classes of resident oyster populations will be necessary. Although our results indicate a limited ability to predict rates of oyster disarticulation at particular sites, it appears that general inferences can be made based on the size classes of oyster boxes collected and differences in salinity regimens. With sampling of natural oyster populations at the various sites evaluated, it should be possible to derive disarticulation rates that can be used to develop models to more accurately

predict natural mortality rates. Unless validated by such additional studies, the relatively long intervals observed in this study for oyster shell disarticulation and the variability in TSD support the conclusions of Mackin (1961) and Andrews (pers. comm., VIMS) that box count data have only limited value in assessing oyster mortality.

Various factors inherent in our experimental design were problematic, including predator exclusion, variability in sample size of the various treatments as a result of differential effectiveness in mortality induction, oyster shell losses resulting from tray deterioration, physical disturbance, and vandalism. One possible con-

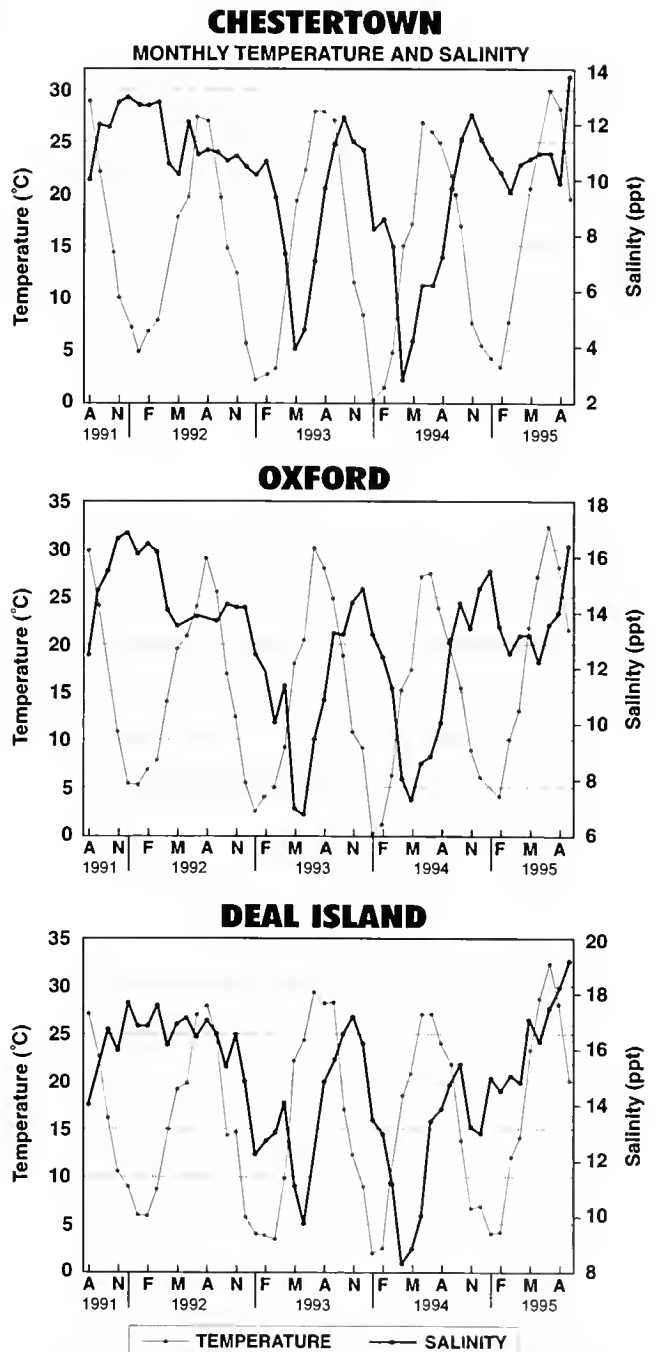


Figure 7. Monthly water temperature and salinity measurements at study sites from 1991 to 1995.

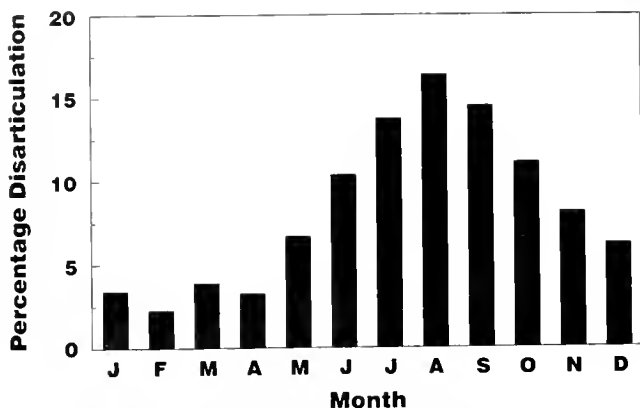


Figure 8. Monthly variation in the cumulative percent disarticulation of oyster shells, with all years, sites, and treatments combined.

foundling factor was that the type of tray used resulted in the exclusion of extremely large predators. As a result, there was a minimization of the physical disturbance resulting from the feeding of predators on the tissue of the gapers, after the sacrifice of the oysters, or during the feeding of predators on epifauna. The injection method of oyster sacrifice, using saturated KCl, had varying degrees of success, with the mortality induced ranging from 65–95%, among treatments. Also, the hardware cloth used to construct the initial trays was subject to rapid deterioration, resulting in substantial losses of oyster shells until the trays were refabricated. At Oxford, physical disturbances caused by storm events resulted in the overturn of trays and the loss of numerous oyster shells from the summer 1991 reef and fall 1991 reef treatments. At Deal Island, vandals partially destroyed the fall 1991 reef tray, which resulted in the loss of the majority of oysters deployed in that treatment.

The absence of effect of substrate type (reef vs. sediment) on TSD may have resulted from the relative similarity of the two substrate types. The reef trays were designed with the intent of simulating the height from the bottom, which an oyster reef would provide, whereas the sediment trays were intended to simulate a situation where oysters were resting directly on the bottom. However, the substrate on which all of the trays were deployed was predominately mud and silt. As a result, even the reef support structures settled into the substrate.

Although the box count method has been commonly used to estimate oyster mortality, because of its low cost and the rapidity of obtaining results, there are two additional methods of using oyster shells to determine TSD: (1) the analysis of radioisotopes and (2) the analysis of free amino acid ratios (Powell et al. 1991). Both methods are costly compared with the box count method but are much more accurate. However, such techniques are not feasible for routine field surveys and are generally used to age articulated oyster shells from much older death assemblages (Powell and Davies 1990).

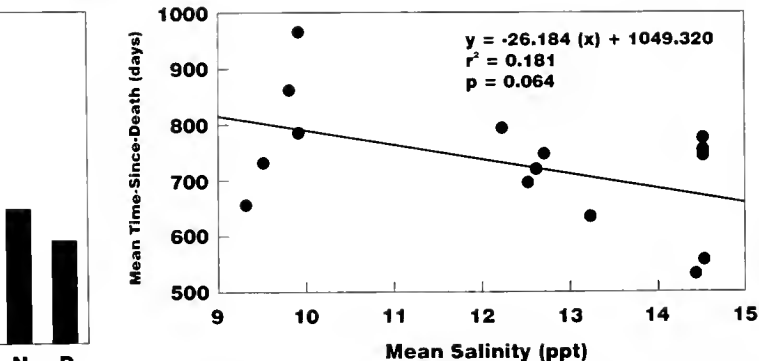


Figure 9. Relationship of mean salinity (ppt) and TSD required for disarticulation of oyster shells deployed in reef treatments (summer, fall, winter, and spring) at each site: Chestertown, Oxford, and Deal Island.

The results of this study indicate that mortality data obtained from box counts should be viewed as a relative indicator of mortality, rather than as an absolute measure, whereas data on live oysters are a direct indicator of the population of live oysters at a particular site. The accuracy of such indirect mortality estimates (Krantz 1992, Krantz 1993, Krantz 1995, Smith and Jordan 1993) depends considerably on the salinity regimen and the time of year in which sampling is conducted. Fall sampling should minimize the possibility of including oyster boxes from previous years in box counts, based on the cumulative monthly disarticulation patterns observed in this study. When interpreting such mortality data, it must be realized that box count data will not necessarily reflect only mortalities that have occurred during the previous year, but will quite probably also reflect mortalities that have occurred during the preceding 2- to 4-y period. In fact, on the basis of the patterns of cumulative percent disarticulation that were observed in this study, mortalities occurring in a given year may be 30–50% less than those estimated on the basis of box count data and the assumption of disarticulation occurring within 1 y after the death of an oyster.

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SETTLEMENT SITE SELECTION BY OYSTER LARVAE, *CRASSOSTREA VIRGINICA*: EVIDENCE FOR GEOTAXIS

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ABSTRACT Settlement of larval oysters, *Crassostrea virginica*, with respect to upper and lower surfaces of natural substrates, was studied in the field and in the laboratory. Enclosures were used to retain pediveligers of *Crassostrea* under controlled field conditions, until they settled. About 62% of these larvae settled onto rough (outer) surfaces of natural oyster shell substrate; this closely matched the proportion of substrate oriented with the rough surface downward. In the laboratory, about 83% of larvae settled onto the lower surfaces of similar shell substrates, in the absence of light, regardless of how the shell substrate was positioned. Both field and laboratory results suggest that geotaxis is a stronger settlement orientation cue than either phototaxis or rugotaxis, in *Crassostrea*.

KEY WORDS: *Crassostrea virginica*, larvae, settlement, geotaxis

INTRODUCTION

Oyster larvae (Ostreidae) have long been studied as a model of bivalve settlement processes but are in fact nearly unique among the more than 90 bivalve molluscan families. Oysters cement permanently to the substrate on selection of a settlement site (Cranfield 1973, Kennedy 1996). In most other bivalve taxa, in contrast, there is at least an epifaunal plantigrade postlarval phase (Carriker 1961, Loosanoff 1961, LaBarbera and Chanley 1971), and for many, there is a planktonic postlarval phase (Sigurdsson et al. 1976, Yankson 1986, Beukema and de Vlas 1989, Martel and Chia 1991). Only for oysters, apparently, is settlement an irrevocable process. What selective advantage this provides is unclear, although some successful nonmolluscan taxa, such as barnacles (Crisp 1961, Le Tourneaux and Bourget 1988) and ascidians (van Duyl et al. 1981), also settle irreversibly. The immediate implications are clear, however—an oyster larva that chooses poorly is doomed.

It is not surprising, therefore, that oyster larvae have exhibited clear responses to environmental cues during settlement. Settlement may be facilitated by water-soluble chemical cues from conspecifics (Crisp 1967, Vietch and Hidu 1971, Coon et al. 1985, Shpigel et al. 1989, Bonar et al. 1990) or bacterial films (Fitt et al. 1990).

Physical cues may also be important. Ritchie and Menzel (1969) reported negative phototaxis in settling *Crassostrea virginica*, in the laboratory. In the natural environment, this behavior would result in highest settlement onto the lower, shaded surfaces of shells of adult oysters, which contain chemical settlement inducers (above). In this orientation, the juvenile oyster would experience a lower risk of burial by sedimentation events, in estuarine environments. Although it has not been tested for oysters, negative geotaxis while settling (often not clearly separable from movement in response to pressure, or barokinesis) could produce similar settlement orientation patterns (Crisp and Ghobashy 1971, Mann and Wolf 1983, Pires and Woollacott 1983).

Substrate texture has been suggested to be a settlement cue for some nonoyster taxa, including shipworms (Dons 1944), brachiopods (Wisely 1969), and barnacles (Wetthey 1986). There are two scales of surface texture on an oyster shell, a common substrate for

settling *Crassostrea*. One is the very large-scale texture (compared with a 300- μ m larva) of shell concavity, with inner surfaces generally being more concave than outer surfaces, and the other is the small-scale rugosity of the outer shell surface, compared with the smooth inner surface (Carriker 1996). Unlike most bivalves, both the inner and the outer surfaces of *Crassostrea* shells are foliated calcite, although some prismatic calcite remains on the outer surfaces of younger oyster shells (Carriker 1996). Rugosity, therefore, is the primary difference between inner and outer shell layers.

Field observations of oyster settlement have often been contradictory. Bonnot (1937) reported that *Ostrea lurida* recruited more on upper surfaces of artificial substrates, but Hopkins (1935) reported the opposite, for the same species. Cole and Knight-Jones (1949) reviewed early literature, which generally reported higher oyster recruitment on lower surfaces. This is in contrast to their own studies (Cole and Knight-Jones 1949), in which they reported higher settlement for *Ostrea edulis* on upper surfaces, when shaded from above (although their results are not significant at $\alpha = 0.05$; this author, reanalysis). Shaw (1967) examined settlement by *C. virginica* onto facing surfaces of paired plates, suspended horizontally. When the plates were 10 cm apart, settlement was higher on the surface facing downward, but placing the plates 2.5 cm apart reversed this trend. However, this trend was not statistically analyzed, nor was variance reported, so the validity of these results cannot be assessed.

Most prior studies of oyster settlement orientation fail to address actual settlement patterns in the field onto natural substrates—typically, the shells of adult conspecifics. This study examined settlement data of a cohort of *C. virginica* onto shells of conspecifics, in a larval enclosure deployed in the field. The field data were compared with settlement patterns onto similar substrate, observed in the laboratory.

MATERIALS AND METHODS

Oyster settlement was observed within larval enclosures placed in the field in the York River estuary, Chesapeake Bay, VA. Larval enclosures were larger than, but similar in principle to, the enclosures used by Young and Chia (1982). Each enclosure was 1 m on a side and 15 cm deep and was covered with snug but removable lids. All construction was clear, 6.5-mm Plexiglas acrylic, and the lid was perforated with four 40-mm circular holes, covered with 150- μ m mesh size to permit water exchange. An input port in the

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center of the lid, to permit the injection of larvae, was fitted with a screw-on cap (Fig. 1).

Dead oyster (*C. virginica*) shells from a York River reef were defaunated by air exposure for several months. Oyster shells have a rough, usually convex outer surface and a smooth, concave inner surface. A 5- to 10-cm layer of defaunated shells was placed into each of four enclosures, at 1.5-m mean tidal depth, and allowed to shift via wave sorting for 24 h. Lids were left off of the enclosures for this period.

C. virginica pediveliger larvae were in the Virginia Institute of Marine Science oyster hatchery. Several hours before larvae were used, they were stained by neutral red stain, which was introduced to the larval water to a concentration of about 10 ppm. Neutral red, a vital stain, has been shown to have little or no harmful effects on oyster larvae but stains larvae for at least several days postsettlement, facilitating sampling (Baker 1991). Enclosure lids were put in place, and approximately equal aliquots (about 110,000 larvae each) were introduced to each enclosure by a diver via lengths of stoppered tubing, through the central input port.

After 24 h, the enclosure lids were removed, and a 10 × 10 grid was placed over each enclosure. Three 10 × 10 cm random samples of shell substrate were removed from each enclosure; edges of the enclosures were not sampled, to avoid possible edge effects. Shell substrates that overlapped grids were assigned to the grid that contained the largest proportion of that shell.

Data on shell substrate orientation in the field samples were not available because of handling techniques; therefore, a separate estimate of substrate orientation was made subsequent to the field sampling (below). The orientations of the rough (outer) surfaces of relatively intact substrate shells were recorded from four separate samples (one random sample from each enclosure), of 15–23 shells each. Grid points previously sampled were avoided. Each orientation was scored as rough surface upward or downward. A goodness-of-fit χ^2 test (Zar 1996) was used to test the null hypothesis that the orientation pattern did not differ from random (50% with rough surfaces facing primarily up, excluding shells lying on edge).

Shell substrates from the field were examined under a dissecting microscope, and juvenile *Crassostrea* were recorded. The numbers of juveniles on rough (outer) and smooth (inner) surfaces

of large, relatively intact shell surfaces were recorded and expressed as proportions; juveniles not clearly on one surface or the other were not included in the analysis. A one-sample *t*-test (Zar 1996) was used to examine the null hypotheses that the proportion of juveniles on rough surfaces did not differ from the observed proportion of shells in the field enclosures with downward-oriented rough surfaces. Proportion data were normalized with an arcsine-square root transformed before analysis (Zar 1996).

A laboratory settlement assay was used to examine settlement onto rough and smooth surfaces of defaunated adult *Crassostrea* shells, with shells in differing orientations. Intact lower valves (the deeply cupped valve that attaches to the substrate), from the same source as shells for the enclosures, were conditioned for 1 day in flume seawater, at the same time as the field enclosures were being prepared. Conditioning permits bacterial growth, which enhances *Crassostrea* larval settlement (Fitt et al. 1990). One shell substrate was placed individually into each of 12 chambers, with 1 L of 150- μ m filtered York River water, in a laboratory at ambient temperature (mean = 29°C). The shell substrate positions were alternated, so that half had the rough surface facing up, and half were facing down. Approximately 500 stained *Crassostrea* larvae from the same cohort used in field enclosures (above) were placed into each chamber. Chambers were covered with black fabric, in a darkened laboratory, for the duration of the trial. After 24 h, *Crassostrea* juveniles settled onto upper and lower surfaces were recorded by use of a dissecting microscope. Two-factor analysis of variance (Zar 1996) was used to test the null hypothesis of no larval orientation (choice of upper or lower surface of shell) or "rugosity" preference (choice of settlement on rough outer surfaces vs. smooth inner surface of shell).

RESULTS

Defaunated oyster shells in the field enclosures tended to lie with the rough surface oriented downward (mean percent oriented downward = 63.9%, SD = 5.0%, $\chi^2 = 68.6$), which differed significantly from random at $\alpha = 0.05$. Approximately 1% of shells in the enclosures were on edge, with no clear upward or downward orientation.

Variability of total settlement between samples was high (mean juveniles per sample = 1.791, SD = 2.284). The proportions of larvae that settled on a particular surface, however, had a much lower variance; the mean proportion on rough surfaces of shell substrates was 61.8% (SD = 15.5%), which did not significantly differ from 63.9%, the observed proportion of shells in the field in which the rough surface was oriented downward ($p = 0.71$). However, the proportion settled on rough versus smooth surfaces did significantly differ from random, or 50% on either surface ($p = 0.0017$).

Settling *Crassostrea* larvae in the laboratory significantly favored the lower surfaces of shells, regardless of whether the rough surface was up or down ($p < 0.0005$), but did not favor smooth surfaces to rough surfaces ($p = 0.66$). The mean proportion of the original cohort that settled on any surface was 64.6% (SD = 16.7%) in 24 h. A mean of 250 juveniles per chamber settled on upper and lower shell substrate surfaces combined; only 3.2 per chamber settled on the edges of the shell substrates, while 70 per chamber settled on the sides of the dishes themselves. Of those larvae that settled on the shell substrates (except for edge specimens), 83.3% (SD = 8.42) settled on lower surfaces, regardless of whether the rough surface of the shell substrate was oriented up-

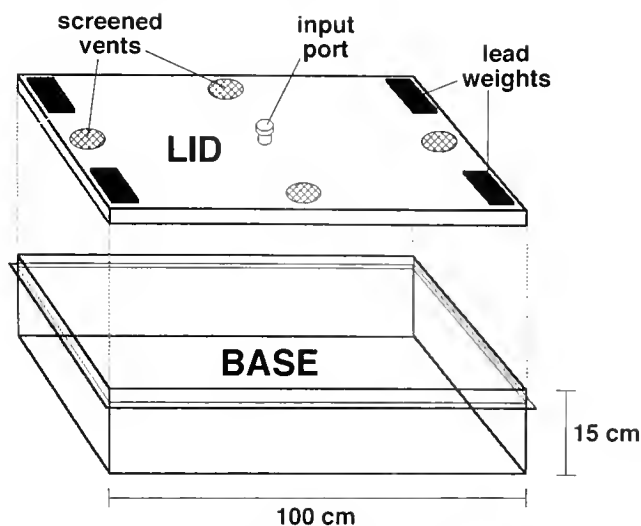


Figure 1. Field settlement enclosure design.

TABLE 1.

Summary of two-factor analysis of variance of the effects of shell substrate orientation (upper or lower shell substrate surface) and rugosity (smooth vs. rough shell substrate surface) on proportional settlement of *Crassostrea* larvae.

Source of Error	df	SS	MS	F	p
Orientation	1	1.69279	1.69279	113.65	<0.0005
Rugosity	1	0.00310	0.00310	0.21	0.660
Interaction	1	0.01636	0.01636	1.10	0.325
Error	8	0.11916	0.01490		
Total	11	1.83141			

DF, degrees of freedom; SS, sums of squares; MS, means squared.

ward or downward. Results of the two-factor analysis of variance on larval settlement site choice are summarized in Table 1.

DISCUSSION

It can be strongly inferred from both field and laboratory data that *Crassostrea* prefers to settle on lower substrate surfaces. This suggests that if pediveliger larvae locate the upper surface of a substrate, they either swim or crawl considerable distances to try to find a lower surface (consider the 70- to 100-mm width of an adult *Crassostrea* shell compared with the 300 + μ m body length of a pediveliger). In the field, this settlement behavior could have been accounted for by differing light levels between upper and lower shell substrate surfaces, as reported by Ritchie and Menzel (1969), but the absence of light in the laboratory trial precluded light as the dominant cue in this study. Barokinesis, or swimming in response to barometric pressure, has been demonstrated for nonestuarine bivalve larvae (Mann and Wolf 1983), but even modest waves in shallow water would make barometric pressure unreliable. Gravity, in contrast, is constant, and geotaxis can account for the settlement patterns observed in this study.

There is prior evidence that *Crassostrea* larvae exhibit geotaxis while settling (Crisp and Ghobashy 1971, Pires and Woollacott 1983). Both *Crassostrea* and *Ostrea* pediveligers have been shown to possess pedal statocysts, which are believed to be geosensory (Cranfield 1973, Eble and Scro 1996). The function of bivalve

statocysts is reviewed by Cragg and Nott (1977), who also describe the ultrastructure of scallop (*Pecten*) pediveliger statocysts, which are similar to those in oyster larvae. Pediveliger larvae are strongly negatively buoyant when not swimming (Cragg and Gruffydd 1975, Mann and Wolf 1983), making gravity an important cue even before settling. If geotaxis is the primary cue used during settlement, exploring pediveliger larvae must process the information that (1) the foot is in contact with appropriate settlement substrate, and (2) the body of the larva is either above or below the substrate.

In contrast to the shell orientation results, laboratory data failed to detect a preference by *Crassostrea* larvae for rough, convex outer surfaces of adult oysters versus smooth, concave inner surfaces. In the absence of currents, larvae are unlikely to be able to detect the concavity of an entire oyster shell, which is several orders of magnitude larger than themselves, and it may be that they are unable to detect the finer-scale rugosity found on the rough outer surfaces of all adult *Crassostrea* shells. Alternately, substrate rugosity on that scale may not be an important settlement cue for *Crassostrea* larvae, although it has been shown to be a cue for other taxa under some conditions (Dons 1944, Wisely 1969, Wethey 1986). A healthy oyster reef, for example, may present primarily living oysters as settlement substrate, with no opportunity for a larval settlement choice between smooth internal and rough external portions of adult shells.

The ecological value, for oysters, of settling on the lower surfaces of substrates is clear. Most *Crassostrea* populations occur in estuaries, which have a high sediment load. In addition, oysters settle gregariously (Crisp 1967, Vietch and Hidu 1971, Coon et al. 1985, Shpigel et al. 1989, Bonar et al. 1990), and adult oysters produce copious feces and pseudofeces, which enhance local sedimentation. Death by siltation is thus a very real possibility to the early juvenile oyster. If a lower surface of a substrate is free, however, it suspended slightly above the sediment and is free from at least normal sedimentation. Chemical cues could help a pediveliger larva locate a reef of conspecifics but would be of little use for selecting lower versus upper surfaces. Other cues could conceivably permit a pediveliger larva to select a site, but on the basis of this study, it appears that geotaxis by itself can account for observed patterns of settlement and that gravity is the primary cue when choosing the final settlement site.

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GONADAL CYCLE OF PEARL OYSTER, *PINCTADA FUCATA* (GOULD) IN NORTHEAST PERSIAN GULF, IRAN

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ABSTRACT The annual cycle of gonad development and spawning in the pearl oyster, *Pinctada fucata* (Gould), in Nakhiloo, Northeast Persian Gulf, was investigated over the 15-mo period from November 1994 to January 1996. Gonadal condition was assessed from criteria developed from histological preparations of randomly collected individuals of all sizes to describe gametogenic development. Gonadal development and spawning trends were similar in both sexes. A bimodal gametogenic pattern with summer and autumn spawning periods occurred. Gametogenesis commenced in November to December, followed by major gonadal maturation during February to April. Summer spawning was observed from April to July, with major spawning at the latter end. During the spawning peak in July, low levels of gametogenesis were noticed. Gametogenic activity increased in August to September, followed by autumn spawning from September to December. Toward the end of the spawning season, the incidence of gonadal inactivation increased. Minimum levels of gonadal activity were observed in November. Temperature regimen appears to have an influential role in the regulation of gametogenic and spawning processes. *P. fucata* was found to be a protandrous hermaphrodite that matured as a male at shell height greater than 20 mm. Bisexuality was uncommon, and the sex ratio was near 1:1.

KEY WORDS: Gonadal cycle, gametogenesis, *Pinctada fucata*, Persian Gulf

INTRODUCTION

Having prospered for more than two thousand years, the pearls of the Persian Gulf have been famous and valuable and praised for their excellent shape and quality. From 1930s onward, with the introduction of Japanese cultured pearls and finally the discovery of the oil in the region, most artisanal pearl fishermen moved from the pearl industry to more lucrative oil-related positions. However, the introduction of modern diving equipment such as air compressors and speed boats, as well as recent developments in pearl farming, has reviewed the pearl industry of the region. At present, the pearl oyster, *Pinctada fucata*, forms the basis for artisanal fisheries and recent development of pearl farming along Iranian coasts of the Persian Gulf.

There is little literature available on the various aspects of the biology and aquaculture of pearl oysters in the northern part of the Persian Gulf. However, elsewhere, *P. fucata* and other species of the *Pinctada* genus have been studied because of their cultivation potential (Tranter 1958; Alagarswami et al. 1983; Gervis and Sims 1992). This study was undertaken to provide baseline information pertaining to the reproductive cycle of *P. fucata* in Nakhiloo, one of the major fishing grounds for pearl oysters along the northeast Persian Gulf. The main objectives of this study were to describe: (1) gametogenesis and spawning period of *P. fucata* in northeast Persian Gulf; (2) influence of environmental parameters on gonadal function and regulation; and (3) population sexuality. The results are intended to be used for management guidelines to maximize annual recruitment of the population and to determine the

time frame when mariculturists can obtain preconditioned broodstock from the natural population, thus reducing hatchery costs.

MATERIALS AND METHODS

Sampling

Samples of oysters covering all size groups were collected monthly from an oyster bed at Nakhiloo (53°22'E, 26°49'N) using SCUBA at four randomly placed quadrats (0.5 × 0.5 m) per month from November 1994 to January 1996. In the laboratory, each oyster was measured along its height (dorsoventral measurement or DVM) to the nearest millimeter with a vernier caliper. A random subsample of about 50 individuals per size group (ranging from 1 to 90 mm DVM at 10-mm class intervals with five individuals per size class, if applicable) was taken for macroscopic and histological examination. Sex and stage of gonadal maturity were examined from fresh smears of gonad. The remaining collected oysters, if any, were measured, dissected, and sexed for sex ratio analysis.

Gonadal Analysis

Having noted the external appearance of gonads enveloping the visceral mass, a piece of gonadal tissue (~1 cm) under the midregion of the mantle lobe was removed, fixed in Bouin's fixative for 24 h, routinely processed, sectioned (5 µm) and stained with Mayer's hematoxylin and eosin. On the basis of the macroscopic and histological examination of gonads, the following stages were recognized for female and male (this classification is based on Rose et al. 1990).

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Early Gametogenesis: Gonadal Tissue Not Visible Macroscopically.

Histologically, follicles appear as scattered patches. For females, the follicular wall is lined with oogonia and developing oocytes (Fig. 1a). For males, there are darkly stained spermatogonia and few visible spermatocytes (Fig. 2a).

Midgametogenesis: Body Incompletely Covered by Gonad at Various Extremities

Some moderately active sperm and club-shaped oocytes are visible. Follicles are enlarged in size, and less connective tissue was seen. For females, the lumen is filling with growing oocytes and some ripe oocytes are present. The follicular wall is sur-

rounded mainly by large elongated oocytes, some showing both nucleus and nucleolus (Fig. 1b). For males, spermatocytes and spermatids are present in increasing number. A few spermatozoa appear in lumen; the pink line of the sperm tails radiates to the center of the lumen (Fig. 2b).

Late Gametogenesis: Gonads Fully Ripe or Near Ripe, Swollen and Almost Completely Covering the Visceral Mass

Gametes ooze free when the gonad is punctured. Earlier stages of gametogenesis are reduced, and densely filled follicles (with gametes) are closely packed. Little or no connective tissue can be observed. For females, the follicles are filled with vitellogenic oocytes, and the lumen of follicles is occupied with both free and

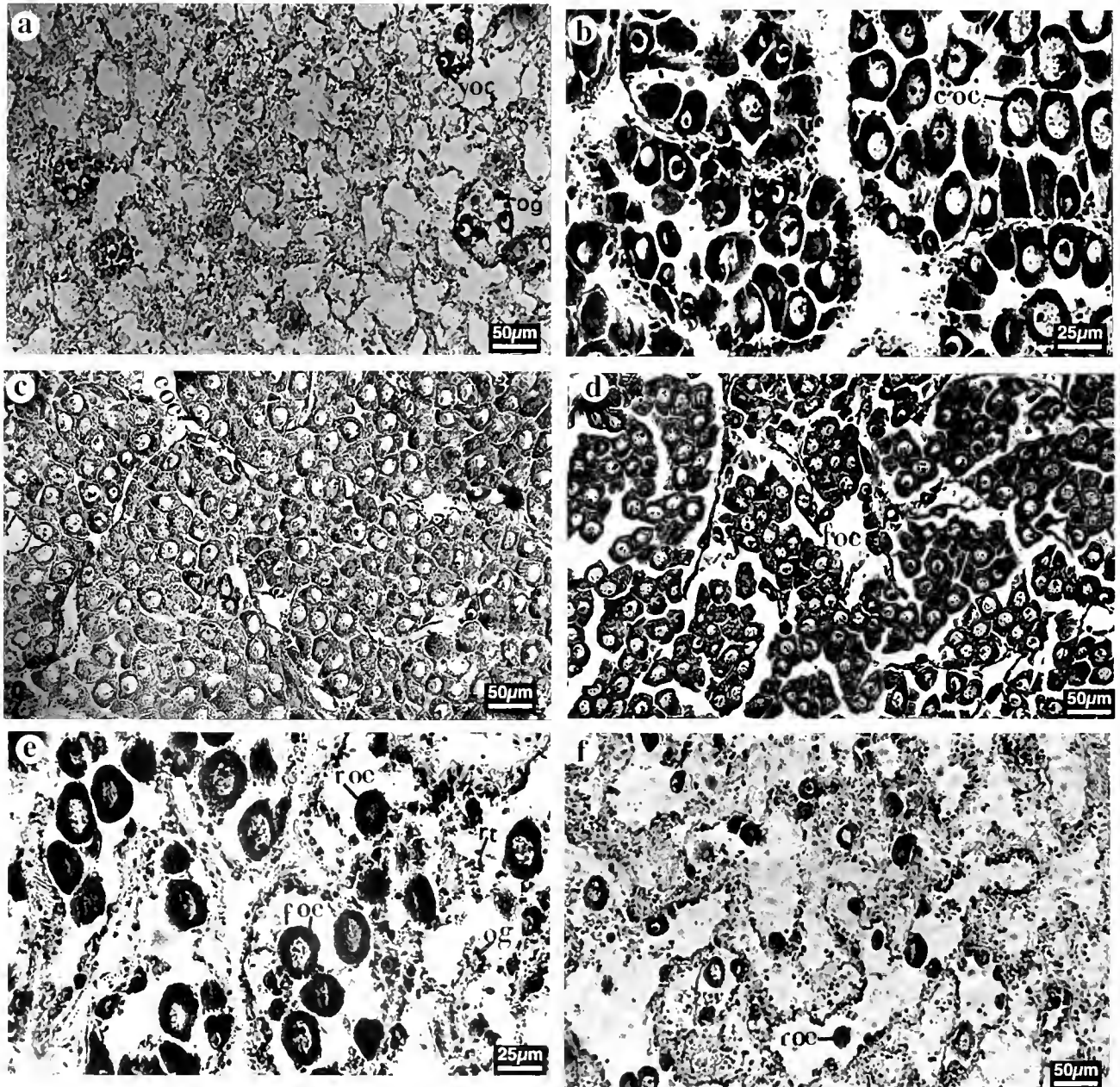


Figure 1. Stages of female gametogenesis in *P. fucata*. (a) Early gametogenesis: follicles showing oogonia (og) and young oocytes (yoc). (b) Midgametogenesis: connected oocytes (coc) beginning to fill the follicular lumen. (c) Late gametogenesis and confluent follicles: lumen of follicles occupied with both free (foc) and connected oocytes. (d) Spawning ovary: confluent follicles with free oocytes and resorptive tissue (rt). (e and f) Spent ovary: follicular lumen occupied by residual oocytes (roc) and resorptive tissue.

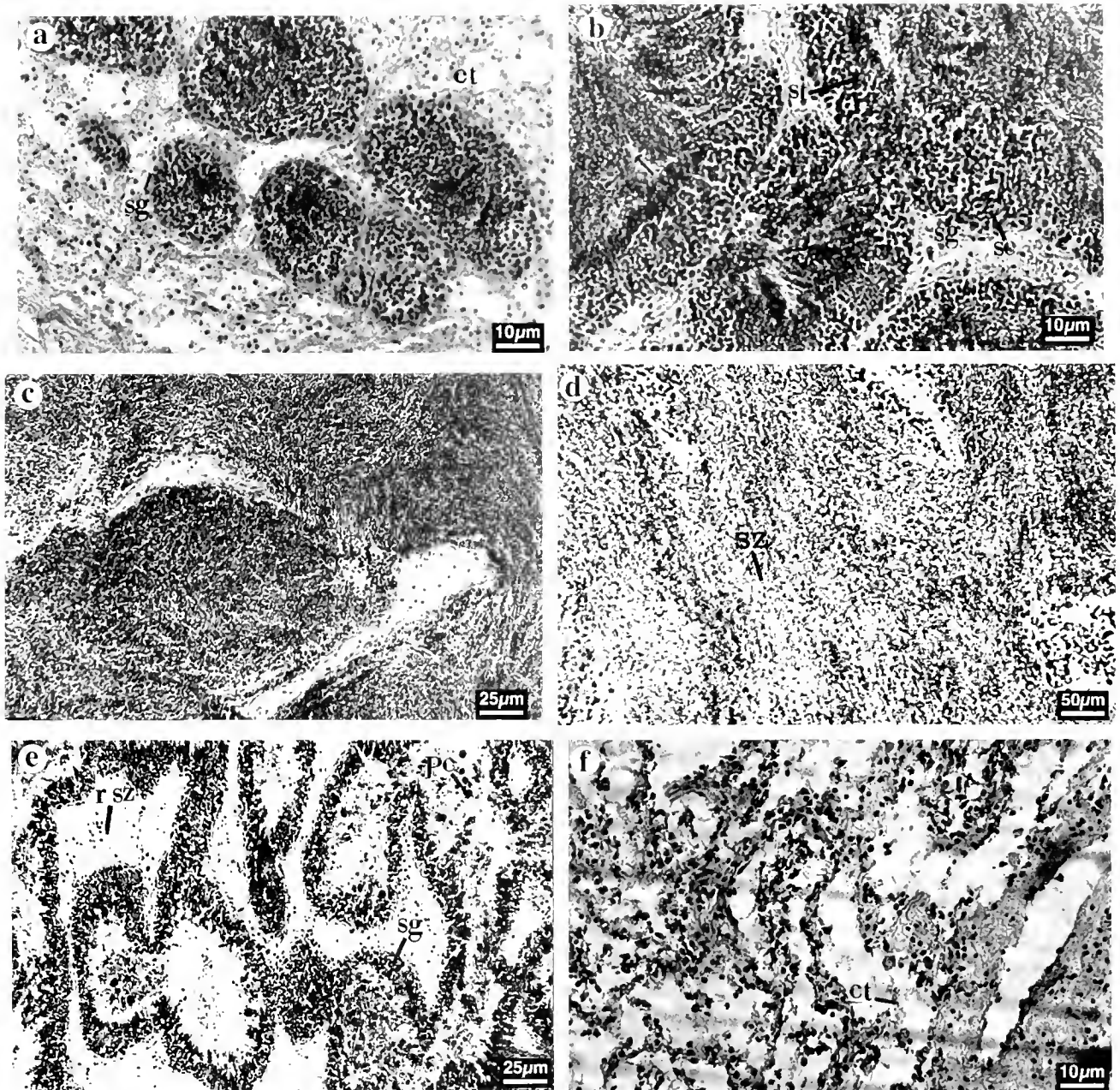


Figure 2. Indeterminate and various stages of male gametogenesis in *P. fucata*. (a) Early gametogenesis: a few follicles with spermatogonia (sg) and considerable connective tissue (ct). (b) Midgametogenesis: follicular wall filled with spermatogonia and developing spermatocytes (sc) and spermatids (st); some follicles showing tail of sperm (arrows). (c) Late gametogenesis: follicular wall filled with developing sperm as a dark band peripherally. (d) Spawning testis: confluent follicles with spermatozoa (sz). (e) Spent testis: follicular lumen showing residual sperm (rsz) and phagocytes (pc); redevelopment occurring along the inside walls of follicles. (f) Indeterminate stage with no sign of gametogenesis.

connected oocytes (polygonal compacted ova) (Fig. 1c). For males, there are very few undifferentiated spermatogenic cells. Follicles are distended and filled with developing sperm, appearing as a dark blue band around the follicular wall, which has decreased in thickness (Fig. 2c).

Spawning

The macroscopic appearance indicates a flaccid gonad with varying amounts of fluid accumulating near the labial palps. The digestive gland is partly visible; underlying gonad and gametes do

not ooze freely when the gonad is punctured. For females, some follicles are almost entirely filled with free oocytes, whereas others are partially spawned, with the appearance of a small amount of resorptive tissue within the follicular lumen that is slightly occupied by residual oocytes. Redeveloping oögonia lined the follicular walls of the empty or near-empty follicles (Fig. 1d). For males, confluent follicles were almost entirely filled with spermatozoa by gaps between the follicular wall and spermatozoa in the lumen. Some follicles have partially empty lumen with phagocytes and a few spermatozoa (Fig. 2d).

Spent

Gonads are considerably shrunken in volume and contain little liquid and a small number of gametes. Spent follicles are empty, except for residual gametes and phagocytes in the lumen. For females, there is a spent ovary showing practically empty follicles filled with resorptive tissue and a few residual free oocytes. No sign of gametogenesis had been seen in spent follicles. Connective tissue begins to reappear (Fig. 1e and f). For males, the follicles are collapsed and decreased in size. A few follicles contain a small amount of residual unspent spermatozoa. Spent testis show phagocytes and redevelopment occurring along the inside walls of the follicles (Fig. 2e).

Indeterminate

No gonadal tissue or gametes were visible. A considerable amount of connective tissue is present (Fig. 2f).

Bisexual Phase

Two forms of a rare sexual condition were observed. One showed both sexes developing together in the same follicles (Fig. 3a). The second form showed one sexual phase overlapping with the other, so residual gametes of the old phase overlapped with growing gametes of the new phase (Fig. 3b).

To facilitate comparison of monthly samples, the mean gonadal index (MGI) was determined by multiplying the number of individuals at each stage by a factor representing the arbitrary rating of the stage. The sum of these products was then divided by the total number of individuals in the sample. The gametogenic stages were assigned ratings as follows: indeterminate = 0, early gametogenesis = 2, midgametogenesis = 3, late gametogenesis = 4, spawning = 2, and spent = 1.

The index may vary from zero, when the entire sample of population is in indeterminate stages, to four, when all are at late gametogenesis. An increase in the MGI indicates progressive development or active gametogenesis, whereas a decline corresponds to spawning and postspawning events. Environmental parameters (water temperature, salinity, pH, and dissolved oxygen) were recorded monthly at a depth range of 7–10 m where oysters were collected.

RESULTS

Sexuality and Overall Sex Ratio

Visual and histological examination of 2,650 oysters revealed that males sexually matured first at a shell size of 20–30 mm

(DVM). Female development was observed at a larger shell size (>25 mm DVM). The overall male/female ratio for 2,650 oysters was 1.05, and the null hypothesis of an equal number of males and females was accepted ($t = 1.10$, $p = 0.05$, $df = 18$). The shell height-frequency distribution of oysters of both sexes is shown in Figure 4. Functional hermaphroditism was uncommon and was observed in only 0.5% of oysters.

Reproductive Periodicity

Monthly assessment of reproductive condition as percentage of various gonadal stages and MGI during the course of study is illustrated in Figures 5 and 6. A bimodal annual gametogenic cycle with summer and autumn spawning periods was identified. Sharp rises in early gametogenesis (males = 7.8%; females = 19.5%) and midgametogenesis (males = 27.3%; females = 31.8%) were observed from November to December 1994. This coincided with an MGI change from 1.25 to 2.00 during same period. During December 1994 to February 1995, a major portion of males (range, 78.9–85%) and females (range, 60–84.5%) were collectively in early and midstages of gametogenesis. A major period of gonadal maturation took place in February to April, when the dominant reproductive stage changed from early and midgametogenesis (males = 80.9%; females = 77%) in February to late gametogenesis (males = 55%; females = 65%) in April (Fig. 5). MGI rose from 2.50 to 3.45 during the same period (Fig. 6). This was followed by summer spawning from April to July. Spawning intensity was relatively light from April to June (approximately 15 to 20% of population) but increased rapidly during July, when a major spawning was observed (males = 50%; females = 66.6%). A decline in MGI was noticed from 3.45 (in April) to 1.85 (in July) during spawning. Following the July peak, spawning declined rather sharply in both sexes in August—about 10–15% of the oyster population were found spawning. Interestingly, during the July spawning peak, a simultaneous sharp decline in all stages of gametogenesis (early to late) was observed (males = 77 to 23%; females = 73 to 0%).

A new gametogenic period of lesser intensity was detected by a rise in early gametogenesis (males = 0–5%; females = 0–5%), midgametogenesis (males = 18.2–40%; female = 0–15%), and late gametogenesis (males = 4.5–35%; females = 0–30%) from July to August, which corresponded to an MGI increase from 1.85 to 2.70. This rise in gametogenesis was preceded by autumn spawning from September to December. Autumn spawning showed moderate to strong intensities, where 32–53% and 44–50% of males and females were spawning, respectively.

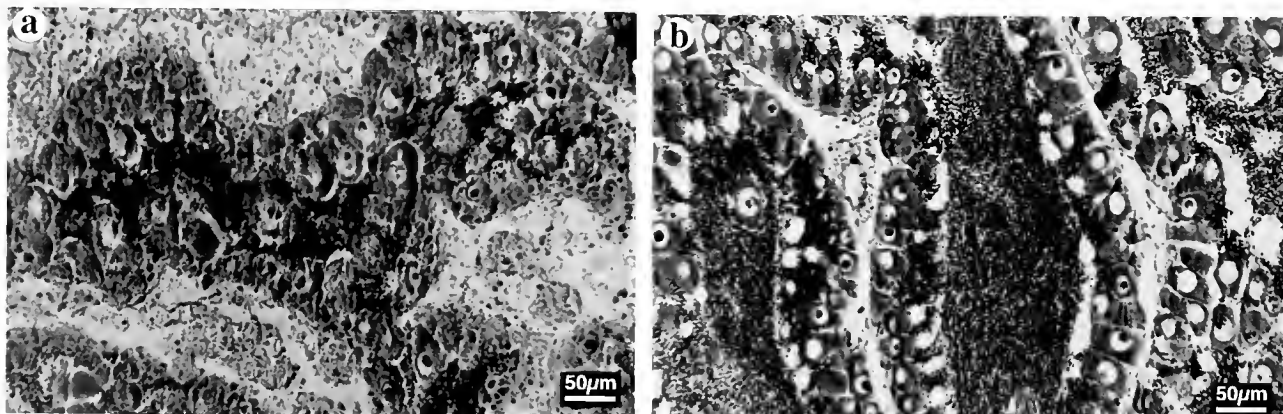


Figure 3. Bisexual phase in *P. fucata*. (a) In the same follicles, both sexes are developed with oocytes surrounding sperm. (b) Developing oocytes in periphery of follicular wall overlapped with residual sperm in follicular lumen.

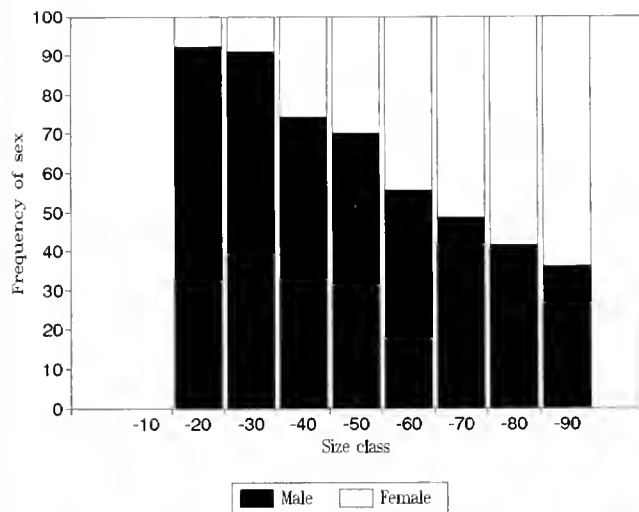


Figure 4. Percentage of height frequency distributions of sexes for populations of *P. fucata* at Nakhiloo.

The incidence of spent individuals rose gradually from July and reached its maximum value in November (males = 47%; females = 54%). Similarly, the indeterminate stage also revealed an increase from October and obtained its highest level in November 1994 and 1995 (Fig. 6). Sharp rises in early and midgametogenesis, which coincided with a moderate rise in late gametogenesis as well as an MGI change from 1.98 to 2.25 during December 1995 to January 1996, were noticed, which indicates the commencement of a new annual cycle. Similar gametogenic and spawning trends were observed for both sexes (Fig. 5).

Environmental Features

Temporal variations in environmental parameters during the course of the study are presented in Table 1. Temperature fluctuated the most compared with the other parameters, which remained relatively constant during the study period. The seawater temperature over the oyster bed at Nakhiloo was at its minimum in February 1995 (21.4°C). The rise of temperature from February to April (27.6°C) coincided with gonadal development and a high incidence of ripe individuals. The commencement of summer spawning in April coincided with a rapid temperature rise from 23.5 to 27.6°C from March to April. Despite lacking some data for the summer spawning period, an increasing trend in water temperature can be noticed (27.6°C in April to 31.7°C in June). Conversely, a major part of the autumn spawning period corresponded to a water temperature decline from 32°C in October to 24°C in December.

DISCUSSION

Bivalve molluscs are known to show considerable variation in their reproductive habits, which may be dependent (Stephen 1980, Dinamani 1987, Robinson 1992, Thorarinsdottir 1993) or independent (Braley 1982) of exogenous clues. When individual gametogenic cycles are synchronized, resulting in simultaneous breeding within a species population, exogenous clues are likely to play a major role in the regulation of gonadal functions (Joseph and Madhystha 1982, Joseph and Madhystha 1984).

It is apparent from the data presented here that the *P. fucata*

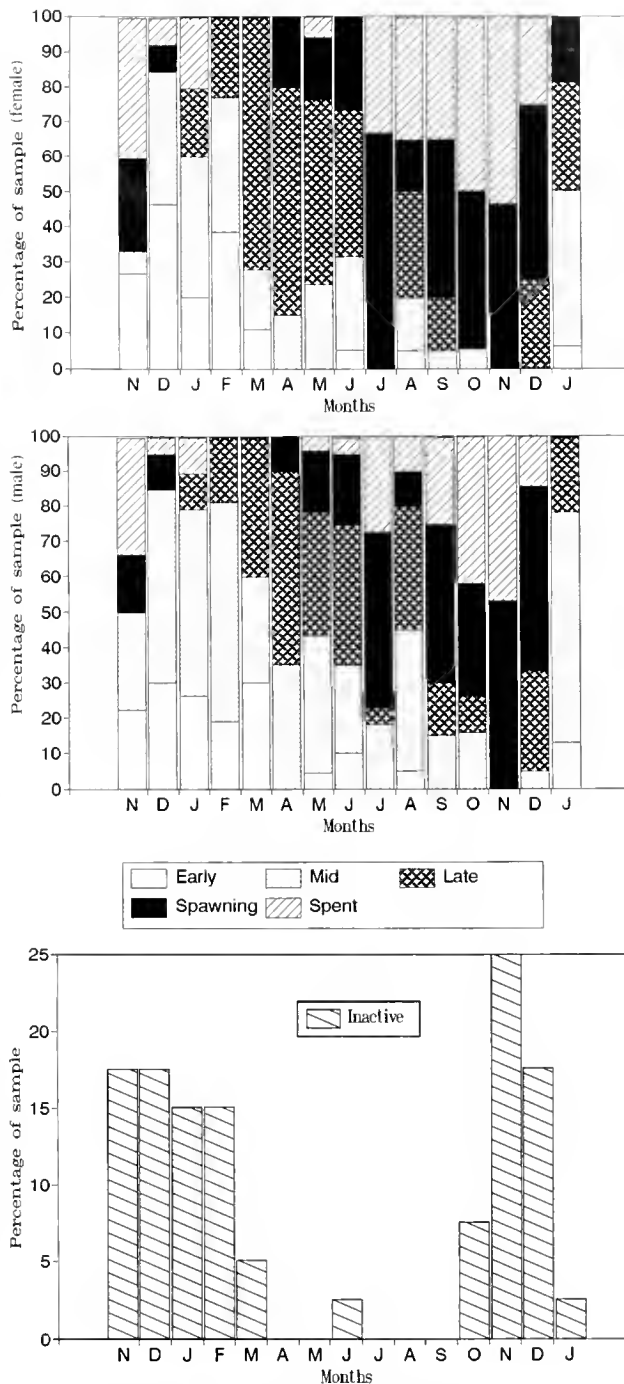


Figure 5. Stages of pearl oyster gonad development derived from histological staging from November 1994 to January 1996.

population in Nakhiloo displayed a synchronized bimodal breeding during summer (April to July) and autumn (September to December). Although it is difficult to specify which exogenous factors are causative agents, the effect of temperature and salinity on the reproductive cycle have been demonstrated for bivalves of the tropics and subtropics (Stephen 1980, Joseph and Madhystha 1982, Joseph and Madhystha 1984, Rose et al. 1990, Baron 1992). In our study area, salinity variations are relatively subtle, indicating minimal influence of this parameter on the gonadal function in *P. fucata*. On the other hand, the proliferation and maturation of

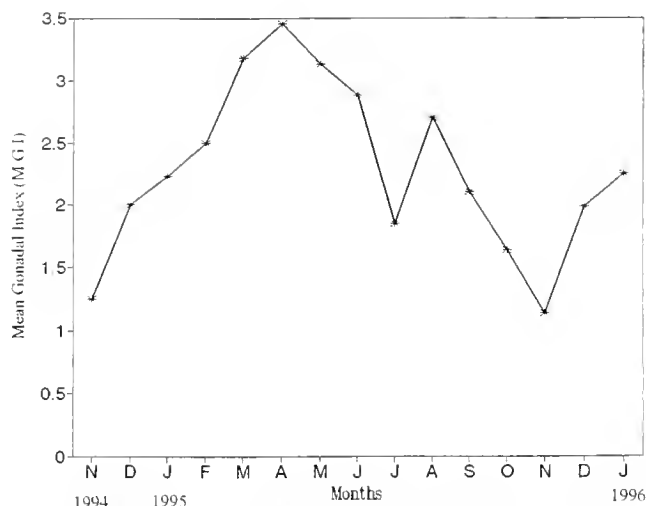


Figure 6. Monthly value of MGI of *P. fucata* in Nakhiloo from November 1994 to January 1996.

gametes during the onset of warm water temperature and their regeneration after the summer spawning period, which also took place during high water temperature, indicate the importance of temperature in the gametogenesis of *P. fucata*.

No published reports exist regarding the gametogenesis of marine bivalves in the Persian Gulf areas, although some aspects of the gonadal development of *Saccostrea cucullata* were reported earlier (Roustaian 1994). In general, the results of this study are in agreement with those of other species of the *Pinctada* genus elsewhere (Tranter 1958, Rose et al. 1990, Gervis and Sims 1992) and that of *S. cucullata* in the Persian Gulf, pointing to the importance of temperature in gametogenesis regulation. However, the extent of gametogenesis dependence on temperature for bivalves and possibly other invertebrates of the Persian Gulf should be approached with caution. It has been shown that an increase in temperature without availability of sufficient quantities of food may result in resorption rather than proliferation of gametes (Véléz and Epifanio 1981). The phytoplankton density of the Persian Gulf has been reported to be minimal during summer (Price 1992, Rezai 1995),

which coincides with active gametogenesis in *P. fucata*. Clearly, to provide a more precise picture on the gametogenic pattern, more research on the possible effect of food supply on reproductive physiology and biochemistry is required. A similar scenario has been described for the mangrove oyster, *Crassostrea rhizophora*, in Venezuela, where the reproductive peak coincides with minimum phytoplankton density during the warm season (Véléz and Epifanio 1981).

Bivalve spawning is often associated with sudden environmental change (Gervis and Sims 1992). In our study, the commencement of summer spawning coincided with a temperature rise of about 4.1°C from March (23.5°C) to April (27.6°C), when spawning was first observed. Although a temperature influence is thought likely, we are prevented from examining this because of a lack of hydrographic data for July. However, previous hydrographic recordings during the last 3 y indicated a maximum or near-to-maximum water temperature during July in the northern part of the Persian Gulf (Rezai 1995). The autumn spawning period (September to December) corresponds with a water temperature change from 32°C in October to 24°C in December. Interestingly, despite the presence of ripe gametes during winter and early spring (March) in the oyster population (13, 18, and 53% in January, February, and March, respectively), no spawning was observed. With these findings in mind, it is hypothesized that temperature fluctuation has a profound influence on the spawning of *P. fucata* in the northern part of the Persian Gulf. Moreover, a critical spawning temperature of around 25°C is speculated for this oyster in the studied area.

Studies on other species of bivalves have revealed a changing reproductive strategy with respect to latitude (Kennedy and Krantz 1982, Heffernan and Walker 1989, Heffernan et al. 1989a, Heffernan et al. 1989b). These studies suggested a change from a rather short unimodal to a prolonged polymodal spawning with decreasing latitude due to an increase in the time over which the critical spawning temperature occurred. The general pattern of spawning in *P. fucata* in the Gulf area reported in this investigation is in line with the findings dealing with oysters in the lower latitudes (Hesselman et al. 1989, Hayes and Menzel 1981).

The results of the study indicate that *P. fucata* is a protandric hermaphrodite. The ability of *P. fucata* to change sex after a certain size appears to be typical of some bivalve molluscs, resulting from unstable genetic sex determination, which can be affected by environmental condition (Mackie 1984).

Because the major portion of the breeding period takes place during the warm season, when food supply may not be at an optimal level, it is speculated that spermatogenesis is favored over more the energy-demanding process of oogenesis at early life stages of the oyster. More insights have yet to be discovered on the ecophysiological significance of protandry with regard to larval survival.

As demonstrated by Lannan et al. (1980) and Kennedy and Krantz (1982) for *Crassostrea gigas* and *Crassostrea virginica*, respectively, the significance of gametogenesis in broodstock management would be the identification of the "optimal window" to maximize larval survival per spawning. On the basis of the result of this study and previous trials on hatchery spawning (Ehteshami and Jahangard, unpubl. data, Persian Gulf Molluscs Research Station), spawning trials and larval rearing are recommended during April to May for *P. fucata*. Moreover, any pearl fishery efforts, if decided to be implemented, are suggested to be conducted after

TABLE 1.

Hydrological data of bottom water over the oyster bed at Nakhiloo, Northeast Persian Gulf, in the period November 1994 to January 1996.

Sampling Date	Temperature (°C)	Salinity (ppt)	Dissolved Oxygen (mg/L)	pH
November 12, 1994	27	36	6.6	8.3
January 11, 1995	22.5	37	5.6	7.9
February 3, 1995	21.4	37	6.6	8.1
March 7, 1995	23.5	37	6.3	7.5
April 21, 1995	27.6	35.8	6.0	8.2
June 19, 1995	31.7	37	5.8	8.1
August 30, 1995	30.5	38.8	—	8.1
October 19, 1995	32	38.5	6.0	8.1
November 10, 1995	28.2	38	5.8	8.0
December 15, 1995	24	34	7.6	7.6
January 10, 1996	23	38	7.8	8.1

summer spawning, to furnish the maximum chance for this valuable species to spawn in the region before commercial harvesting.

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EVALUATION OF MICROENCAPSULATED SQUID OIL AS A SUBSTITUTE FOR LIVE MICROALGAE FED TO PACIFIC OYSTER (*CRASSOSTREA GIGAS*) SPAT

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ABSTRACT The potential of gelatin-acacia microcapsules (GAM) containing squid oil as a replacement for a diet of live microalgae (LMA) consisting of a 1:1 mixture of *Chaetoceros muelleri* and T-ISO was assessed for Pacific oyster (*Crassostrea gigas*) spat. Shell length, dry weight, and ash-free dry weight (AFDW) of spat was negatively correlated with the dietary level of GAM after 28 days. However, shell length, dry weight, and AFDW of spat fed 80% LMA plus 20% GAM were not significantly different from those of spat fed a 100% LMA ration. Furthermore, the nutritional value of GAM was indicated by the significantly higher AFDW of spat fed 80% LMA plus 20% GAM and 60% LMA plus 40% GAM compared with that of spat fed the same LMA ration without GAM supplementation. When 40 and 60% of LMA were replaced by GAM, the increases in AFDW of spat were 89 and 77% that of those fed 100% LMA, respectively. The AFDW of spat fed the 100% GAM diet was significantly higher compared with that of unfed spat. The results show that GAM could potentially replace up to 20% of LMA used in commercial hatcheries without affecting growth rates.

KEY WORDS: Nutrition, squid oil, microcapsule, microalgae, oyster, *Crassostrea gigas*

INTRODUCTION

The production of live microalgae (LMA) as food for bivalve larvae and spat in commercial hatcheries accounts for approximately 30% of operating costs (Coutteau and Sorgeloos 1992). As a consequence, there has been considerable research interest in the development of suitable "off the shelf" diets as replacements for live microalgae. Some of these potential alternatives, such as dried microalgae, microalgal pastes, and microencapsulated or yeast-based artificial diets (for review, see Coutteau and Sorgeloos 1993), have shown promising results. For example, between 50 and 90% of a live microalgal diet could be successfully substituted with the spray-dried flagellate *Tetraselmis suecica* when fed to juvenile clams and oysters (Laing and Millican 1992). Moreover, replacement of up to 50% of a LMA diet by modified baker's yeast (*Saccharomyces cerevisiae*) did not affect the growth of juvenile clams (Coutteau et al. 1994b). Langdon and Siegfried (1984) fed an artificial diet containing microcapsules to oyster spat and obtained growth rates as high as 73% of that of controls fed LMA. Bivalve larvae have also been successfully reared to metamorphosis on microencapsulated diets (Chu et al. 1987); however, growth rates of larvae fed artificial diets are generally low compared with those of larvae fed LMA (Chu et al. 1987, Southgate et al. 1992).

Gelatin-acacia microcapsules (GAM) have been commonly used to present lipids in nutritional studies with bivalves (Langdon and Waldock 1981, Chu et al. 1982, 1987, Southgate 1988, Numaguchi and Nell 1991, Knauer and Southgate 1997a). GAM are readily digested (Chu et al. 1982, Southgate 1988), and lipid supplied in GAM is assimilated with high efficiency (Knauer and Southgate 1997b). Although GAM are not suitable to deliver complete artificial diets to bivalves, Numaguchi and Nell (1991) established that GAM could reduce the requirement for LMA for

Sydney rock oyster (*Saccostrea commercialis*) larvae. However, the potential of GAM as a partial replacement for LMA has not yet been quantified for bivalves.

This study evaluated the potential of GAM containing squid oil as a substitute for LMA fed to Pacific oyster (*Crassostrea gigas*) spat. Squid oil was chosen because it contains high levels of the fatty acids eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) (Numaguchi and Nell 1991, Southgate and Lou 1995, Knauer and Southgate 1997a), which are considered essential for bivalves (Langdon and Waldock 1981, Knauer and Southgate 1997a).

MATERIALS AND METHODS

Diets

The diatom *Chaetoceros muelleri* (code CS 176) and the flagellate *Isochrysis* aff. *galbana* (strain T-ISO, code CS 177) were obtained from CSIRO Marine Laboratories (Hobart, Australia) and cultured as previously described (Knauer and Southgate 1996). The dry weights (DW) of *C. muelleri* (33.9 ± 2.7 pg cell⁻¹) and T-ISO (25.7 ± 3.1 pg cell⁻¹) were determined using the method of Utting (1985).

GAM containing squid oil (Aquafeed Products, Brisbane, Australia) were prepared according to the method of Southgate and Lou (1995). Briefly, 2.5 mL of squid oil was homogenized with a 1:1 mixture of a 2% (w/v) solution of gelatin and a 2% solution of acacia, which were made up separately in distilled water. The pH of the mixture was reduced to 3.7 using dilute HCl, and after stirring for 5 min, the pH was increased to 9.3 by the addition of dilute NaOH. The resulting GAM suspension was poured into an equal volume of distilled water and held in a refrigerator for 2 h. The GAM solution was then centrifuged at 3,000 g for 15 min at 4°C. The GAM were collected by spatula and resuspended in distilled water. A fresh batch of GAM was prepared every 9 days during the experiment. The DW of GAM was determined weekly

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by oven drying triplicate 1-mL volumes of stock suspension. The mean diameter of GAM was $4.7 \pm 2.2 \mu\text{m}$ ($n = 100$).

Three 1-L samples of both *C. muelleri* and T-ISO were centrifuged at 3,000 g for 10 min. The resulting microalgal pastes were washed with 100 mL of 0.5 M ammonium formate, recentrifuged (Brown and Jeffrey 1992), and then oven-dried and stored at -80°C before biochemical analyses. A sample of each of the three batches of GAM was also collected and stored in the same way before analyses. The energy contents of microalgae and GAM samples were determined via bomb calorimetry following the method of Knauer and Southgate (1997a). The protein content of the samples was determined according to the Folin-Lowry method (Lowry et al. 1951). The determination of the lipid content followed a modified version of the sulfuric acid charring method (Marsh and Weinstein 1966), as outlined by Knauer and Southgate (1996). Finally, carbohydrate content was assayed by the procedure of Raymont et al. (1963) after the samples were prepared according to Shibko et al. (1967).

Feeding Experiment

C. gigas spat were obtained from Shellfish Culture P/L (Tasmania, Australia). They were unfed for 1 day, after which 50 randomly selected spat were rinsed with distilled water and measured for initial shell length (SL), DW, and ash-free dry weight (AFDW) as previously described (Knauer and Southgate 1996).

The experiment was randomized with three replicate aquaria per treatment. Each aquarium contained 40 spat held in baskets made of plastic mesh (1-mm diameter). Group wet weights ranged from 1.02 to 1.12 g; the variation from the mean wet weight of all groups (1.07 ± 0.03 g) did not exceed $\pm 5\%$. Aquaria were filled with 4 L of filtered seawater (FSW) (5 μm , 1 μm , 0.45 μm , and activated carbon cartridge filtration, followed by ultraviolet sterilization) with a salinity of 30‰. The water was gently aerated to reduce food sedimentation. Spat were kept under a 12hL:12hD photoperiod at $25.0 \pm 0.6^\circ\text{C}$. The water in all aquaria was changed every 24 h, and every 5 days, the surfaces of each aquarium were sterilized with chlorine solution and washed with freshwater before refilling with FSW. At the same time, the baskets and the oyster spat were cleaned by spraying with FSW.

Spat were fed either LMA composed of a 1:1 mixture of *C. muelleri* and T-ISO (100, 80, or 60% ration), LMA partially replaced with GAM (ratio LMA/GAM: 80/20, 60/40, 40/60, and 20/80%), or 100% GAM. Each aquarium received the same DW ration once daily, which was calculated using the formula of Epifanio (1979):

$$Q_R = 0.01 \times W^{-0.33}$$

where Q_R = the DW of ration per gram wet weight of oysters, and W = gram initial wet weight of oysters. Previous studies have shown that this ration is appropriate for *C. gigas* spat (Knauer and Southgate 1996, Knauer and Southgate 1997a). The spat in a further three aquaria were not fed throughout the experiment. After 28 days, all spat were unfed for 1 day and 20 spat were selected at random from each aquarium to measure SL, DW, and AFDW as described above.

Statistical Analyses

The homogeneity of the variances of means was confirmed using Cochran's test. The proximate compositions and caloric contents of microalgae and GAM, and the growth data were analyzed

using a one-way analysis of variance. Multiple comparisons were made using Tukey's multiple range test. Results were considered to be significantly different at $p \leq 0.05$.

RESULTS

The proximate compositions and energy contents of *C. muelleri*, T-ISO, and GAM are shown in Table 1. T-ISO had a significantly higher protein content ($390.4 \pm 9.0 \mu\text{g mg}^{-1}$) than either *C. muelleri* ($308.1 \pm 12.9 \mu\text{g mg}^{-1}$) or GAM ($44.7 \pm 16.0 \mu\text{g mg}^{-1}$). In contrast, the lipid content of GAM ($838.8 \pm 10.3 \mu\text{g mg}^{-1}$) was significantly higher than that in both *C. muelleri* ($209.8 \pm 7.0 \mu\text{g mg}^{-1}$) and T-ISO ($210.8 \pm 6.1 \mu\text{g mg}^{-1}$). *C. muelleri*, however, contained significantly more carbohydrate ($182.5 \pm 8.5 \mu\text{g mg}^{-1}$) than T-ISO ($77.8 \pm 15.7 \mu\text{g mg}^{-1}$) and GAM ($59.4 \pm 7.6 \mu\text{g mg}^{-1}$). Finally, the energy content of GAM ($33.7 \pm 1.2 \text{ J mg}^{-1}$) was significantly higher than that in the microalgae (*C. muelleri*, $19.2 \pm 1.2 \text{ J mg}^{-1}$; T-ISO, $13.1 \pm 0.7 \text{ J mg}^{-1}$).

The proximate compositions and energy contents of the various experimental diets (Table 2) were calculated on the basis of the data presented in Table 1. An increase in dietary GAM content from 0 to 100% resulted in a decrease in dietary protein from 34.9 to 4.5% and a decrease in dietary carbohydrate from 13.0 to 5.9%. At the same time, dietary lipid increased from 21.0 to 83.9% and total energy content of the diets increased from 16.2 to 33.7 J mg^{-1} .

There were no mortalities of spat in any of the treatments during the experimental period. The SL, DW, and AFDW of *C. gigas* spat fed different ratios of LMA and GAM are shown in Table 3. SL varied from 5.47 ± 0.12 mm in unfed spat to 8.84 ± 0.04 mm in spat fed the 100% ration of LMA. The SL of spat fed an 80% ration of LMA plus 20% GAM (8.79 ± 0.12 mm) did not differ significantly from that of spat fed 100% LMA. However, spat fed either of these diets had a significantly greater SL than spat in all other treatments. There was no significant difference in SL between unfed spat and spat fed 100% GAM (5.79 ± 0.15 mm). The DW of spat fed the 80% ration of LMA plus 20% GAM (57.81 ± 1.18 mg) was not significantly different from the DW of spat fed the 100% LMA diet (60.61 ± 3.00 mg). However, spat fed the 100% LMA diet had a significantly higher DW than those in all other treatment groups, whereas the DW of spat fed 80% LMA plus 20% GAM did not differ significantly from that of spat fed the

TABLE 1.

Proximate compositions and energy contents of *C. muelleri*, T-ISO, and GAM containing squid oil.

Diet	Composition ($\mu\text{g mg}^{-1}$ DW)			Energy Content (J mg^{-1})
	Protein	Lipid	Carbohydrate	
<i>C. muelleri</i>	308.1^b ± 12.9	209.8^b ± 7.0	182.5^a ± 8.5	19.2^b ± 1.2
T-ISO	390.4^a ± 9.0	210.8^b ± 6.1	77.8^b ± 15.7	13.1^c ± 0.7
GAM	44.7^c ± 16.0	838.8^a ± 10.3	59.4^b ± 7.6	33.7^a ± 1.2

Values are the mean \pm SD ($n = 3$). Means in each column with different superscripts are significantly different ($p \leq 0.05$).

TABLE 2.

Proximate compositions and energy contents of diets presented to *C. gigas* spat during a 28-day growth trial.

Diet (%LMA: %GAM)	%DW			Energy Content (J mg ⁻¹)
	Protein	Lipid	Carbohydrate	
100:0	34.9	21.0	13.0	16.2
80:0	34.9	21.0	13.0	16.2
60:0	34.9	21.0	13.0	16.2
80:20	28.8	33.6	11.6	19.7
60:40	22.7	46.2	10.2	23.2
40:60	16.7	58.7	8.7	26.7
20:80	10.6	71.3	7.3	30.2
0:100	4.5	83.9	5.9	33.7

Diets were composed of various proportions of LMA (1:1 mixture of *C. muelleri* and T-ISO) and GAM containing squid oil. The 100% ration of food was 10 mg (DW) per replicate.

80% LMA diet (55.03 ± 2.00 mg). The DW of all fed spat was significantly greater than the DW of unfed spat (17.14 ± 1.23 mg). The AFDW of spat fed the 100% ration of LMA (4.02 ± 0.08 mg) did not differ significantly from that of those fed 80% LMA plus 20% GAM (3.99 ± 0.15 mg), but was significantly greater than that of spat in all other treatments. The AFDW of spat fed the 80% LMA plus 20% GAM diet was significantly higher than that of spat fed an 80% ration of LMA (3.54 ± 0.05 mg), and that of spat fed 60% LMA plus 40% GAM (3.57 ± 0.17 mg) was significantly higher than the AFDW of spat fed a 60% LMA ration only (3.22 ± 0.27 mg). However, the AFDW of spat fed 80% LMA plus 20% GAM was not significantly higher than that of spat fed the 60% LMA plus 40% GAM diet. Moreover, all fed spat had a significantly higher AFDW than unfed spat (0.96 ± 0.07 mg).

The relationship between the dietary content of GAM and the growth of spat is shown in Figure 1. Increasing replacement of microalgae with GAM resulted in a decrease in SL ($r^2 = 0.9555$, $p \leq 0.001$), DW ($r^2 = 0.9823$, $p \leq 0.001$), and AFDW ($r^2 = 0.9334$, $p \leq 0.01$) of *C. gigas* spat.

TABLE 3.

SL, DW, and AFDW of *C. gigas* spat fed mixed diets of LMA (1:1 mixture of *C. muelleri* and T-ISO) and GAM containing squid oil for 28 days.

Diet (%LMA: %GAM)	SL (mm)	DW (mg)	AFDW (mg)
100:0	8.84 ± 0.04^a	60.61 ± 3.00^a	4.02 ± 0.08^a
80:0	8.35 ± 0.22^b	$55.03 \pm 2.00^{b,c}$	3.54 ± 0.05^c
60:0	7.41 ± 0.17^c	46.98 ± 1.58^d	$3.22 \pm 0.27^{c,d}$
80:20	8.79 ± 0.12^a	$57.81 \pm 1.18^{a,b}$	$3.99 \pm 0.15^{a,b}$
60:40	7.52 ± 0.05^c	$50.33 \pm 0.46^{c,d}$	$3.57 \pm 0.17^{b,c}$
40:60	6.88 ± 0.19^d	40.70 ± 2.43^e	3.08 ± 0.07^d
20:80	6.40 ± 0.15^e	32.09 ± 1.40^f	2.26 ± 0.20^e
0:100	5.79 ± 0.15^f	24.11 ± 1.73^g	1.85 ± 0.13^e
Unfed	5.47 ± 0.12^f	17.14 ± 1.23^h	0.96 ± 0.07^f

Values are the mean \pm SD ($n = 3$). Means in each column with different superscripts are significantly different ($p \leq 0.05$). Initial values ($n = 50$): SL, 5.30 ± 0.50 mm; DW, 16.49 ± 4.76 mg; AFDW, 1.00 ± 0.18 mg.

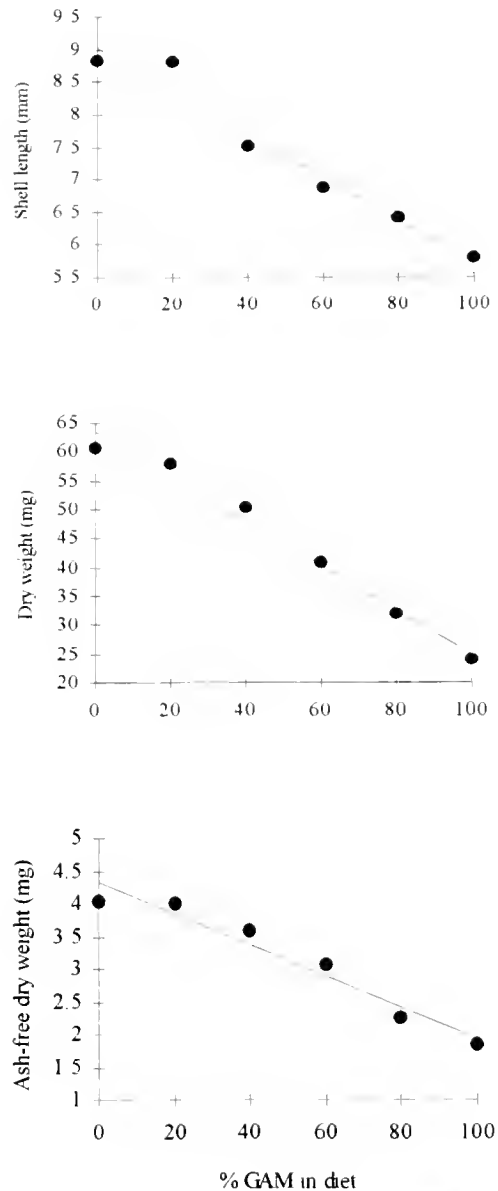


Figure 1. The relationship between percent/dietary GAM and SL ($r^2 = 0.9555$, $p \leq 0.001$), DW ($r^2 = 0.9823$, $p \leq 0.001$), and AFDW ($r^2 = 0.9334$, $p \leq 0.01$) of *C. gigas* spat at the end of a 28-day growth trial.

DISCUSSION

Dietary lipids or lipid components have been experimentally delivered to bivalves using liposomes (Parker and Selivonchick 1986), lipid microspheres (Robinson 1992a,b, Heras et al. 1994), and lipid emulsions (Couteau et al. 1994a, 1996). However, GAM have been more frequently used in nutritional studies with bivalves (Langdon and Waldock 1981, Chu et al. 1982, 1987, Southgate 1988, Numaguchi and Nell 1991, Knauer and Southgate 1997a) and have also been shown to be effective for fatty acid enrichment of *Artemia* (Southgate and Lou 1995). The results of this study support a previous finding that GAM are suitable as a partial substitute for LMA fed to bivalves (Numaguchi and Nell 1991). The replacement of 20% of LMA with GAM did not result in reduced AFDW of *C. gigas* spat when compared with that of spat fed a 100% ration of LMA; however, spat growth was inversely

related to the amount of dietary GAM. Moreover, the AFDW of spat fed 80% LMA plus 20% GAM and 60% LMA plus 40% GAM was significantly higher than that of spat fed the same LMA ration without GAM supplementation. This and the significantly higher AFDW of spat fed 100% GAM diet compared with unfed spat clearly demonstrate that GAM containing squid oil were of nutritional value to *C. gigas* spat. Furthermore, when 40 and 60% of LMA were replaced by GAM, the increases in AFDW of spat were 89 and 77%, respectively, that of spat fed the 100% ration of LMA. This demonstrates that GAM could be a useful supplement if, for some reason, sufficient quantities of LMA are unavailable to feed spat (e.g., Numaguchi and Nell 1991).

Very little is known about the nutritional requirements of bivalves mainly because of the lack of suitable artificial diets in which diet composition can be precisely controlled. The importance of dietary lipids as a supply of energy and essential metabolites for the early life stages of bivalves has been demonstrated (Gabbott and Holland 1972, Holland and Spencer 1973, Gallager and Mann 1986, Gallager et al. 1986), but the requirement for dietary lipids has not yet been determined. Similarly, the importance of an adequate supply of dietary carbohydrates to bivalves has also been emphasized (Haven 1965, Flaak and Epifanio 1978, Whyte et al. 1989), but dietary carbohydrate requirements are still to be quantified. However, the protein requirements of bivalves have been estimated to range from 13% of the DW of the diet for littleneck clam (*Ruditapes decussatus*) spat (Albentosa et al. 1996) to 30 to 60% for larval molluscs in general (Brown et al. 1989). Kreeger and Langdon (1993) demonstrated that the growth of mussel (*Mytilus trossulus*) juveniles fed T-ISO supplemented with various rations of microencapsulated protein could be limited at dietary protein contents of less than 40%. In this study, an increase in the proportion of dietary GAM resulted in an increase in dietary lipid and energy content and a concomitant reduction in dietary protein and carbohydrate content. Spat growth was not significantly affected by replacing 20% of the LMA diet with GAM. This diet contained 29% protein, 34% lipid, and 12% carbohydrate on a DW basis. At levels of microalgal substitution of more than 20%, spat growth significantly declined, indicating that levels of dietary

protein and/or carbohydrate may have become limiting. It should be noted, however, that differential ingestion of LMA and GAM may have occurred, and as such, the nutritional composition of the ingested diet may have differed from that of the diet presented.

The protein, lipid, and carbohydrate content of *C. muelleri* used in this study is similar to previously reported values (Knauer and Southgate 1996). The protein content of T-ISO used in this study falls within the 17–44% range previously reported (Whyte 1987, Albentosa et al. 1996). Likewise, the lipid content of T-ISO is within the 11–28% range reported in prior studies (Wikfors et al. 1992, Albentosa et al. 1993). However, the carbohydrate content of T-ISO used in this study is relatively low compared with that used in other studies, which reported levels of 9–20% (Whyte 1987, Albentosa et al. 1993). No data on the proximate composition of GAM have previously been published; however, the lipid content of the GAM used in this study falls at the lower end of the 81–93% range previously reported (Numaguchi and Nell 1991, Southgate and Lou 1995).

Commercial and research hatcheries consider food value, price, and ease of use as the most important parameters of an "off the shelf" diet (Coutteau and Sorgeloos 1992). Under laboratory conditions, the food values of some spray-dried microalgae (Laing and Millican 1992), microencapsulated diets (Laing 1987, Southgate et al. 1992), and yeasts (Epifanio 1979, Urban and Langdon 1984, Coutteau et al. 1994a, Nell et al. 1996) have been reported to be higher than that of GAM in this study. However, these diets generally perform less well in commercial hatcheries, probably as a result of difficulties in on-site preparation and presentation (Coutteau and Sorgeloos 1992). Although GAM are simple to produce, relatively easy to use, and can be prepared rapidly on demand, the potential of GAM as a substitute for LMA under large-scale culture conditions is still to be assessed.

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OPTIMIZING SUBTIDAL OYSTER PRODUCTION, MARLBOROUGH SOUNDS, NEW ZEALAND: SPIONID POLYCHAETE INFESTATIONS, WATER DEPTH, AND SPAT STUNTING

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ABSTRACT Four species of spionid polychaete of the genus *Boccardia* and three species of the genus *Polydora* were extracted from subtidal Pacific oysters using the vermifuge phenol and di-chlorobenzene, over a 15-mo study in Admiralty Bay, Marlborough Sounds, New Zealand. Shell blistering attributed to the burrowing activities of *Boccardia knoxi* infestations occurred over the second summer of growth after the hanging out of spat at the study sites in December 1993. The infestation period was consistent with a spring dispersive phase of *B. knoxi* larvae. After 13 mo of suspension, the proportion of oysters containing shell blisters was significantly greater at 6- to 12-m depth than near the water surface, which was attributed to the increased shell growth at the surface. Up to 57% of the oysters contained shell blisters, and 17% were unsuitable for the half-shell trade. In a separate experiment, oyster spat were subjected to increasing periods of intertidal storage or "hardening" in order to optimize growth rates and condition of subsequent subtidal cultivations. Spat were stunted by two methods: intertidal hardening and storage in a recirculating tank assumed to be food limited. Spat from each treatment were transferred to two growout sites in the Marlborough Sounds at monthly intervals over 9 mo. Spat stunting up to 9 mo had no significant effect on the minimum size of harvest within each site. After 9 mo of hardening, intertidally stunted spat grew significantly larger than spat stunted in a tank. Increased duration of stunting produced increased subtidal growth, with an almost doubling of growth rates between 1 and 10 mo of stunting. Stunting method or period had no significant effect on condition index or the derivative dry meat and dry shell weights at each site. Strong intersite differences in growth and condition were attributed to hydrodynamic factors. The progressive increase in growth rates with increasing stunting period indicated that the energy-compensatory and energy-conserving mechanisms developed intertidally by *Crassostrea gigas* spat increased with time. Because these adaptive mechanisms were also manifest in submerged spat fed on a limited diet, they appeared to be controlled by food limitation rather than by discontinuous feeding patterns present in the intertidal. An annual crop rotation is suggested for areas prone to *B. knoxi*, and spat stunting for optimal periods can be used to optimize subtidal growth and condition, thus reducing the growout time and avoiding fouling and spionid infestations.

KEY WORDS: Spionid polychaete, Spionidae, *Boccardia*, *Paraboccardia*, *Polydora*, Pacific oyster, *Crassostrea gigas*, shell blisters, aquaculture, subtidal

INTRODUCTION

Wild populations of the Pacific oyster *Crassostrea gigas* spread rapidly throughout the top of the South Island of New Zealand since the first reported sightings in the Marlborough Sounds in 1977 (Jenkins and Meredyth-Young 1979). As a diversification of the dominant Greenshell mussel industry in the Marlborough Sounds, subtidal Pacific oyster culture methods have been adopted, allowing expansion of aquaculture into areas unsuitable for mussel culture. Early longline trials in Admiralty Bay (Fig. 1) produced growth rates competitive with those in the North Island industry (pers. observ.); however, the presence of shell blisters induced by spionid polychaete worm infestations indicated future problems with these pests if the target market was to be the lucrative half-shell trade (Handley 1995).

The method of intertidal storage or "hardening" of oyster spat has been used in the Marlborough Sounds out of necessity to store spat between the overlapping spat catching and growout periods, which allow oysters to be produced on an annual crop rotation. The technique of hardening spat was first developed after mass mor-

talities were experienced in subtidal raft culture of Pacific oysters in Japan during the 1920s (Ventilla 1984, Ogasawara et al. 1962). Ogasawara et al. (1962) found that different hardening periods significantly affected survival, growth rates, spawning, water content of meats, and recovery of condition of oysters after spawning. The significance of hardening spat in the developing Marlborough oyster industry had not been tested. Most of the subtidal oyster production in the Marlborough Sounds to date has relied on wild spat settled on collectors, which are then stored on intertidal racks until longlines become available for hanging out the spat. Wild spat are held in high-density bundles on their collecting substrate in the upper intertidal, at a level and density that stunt their growth. As a result of limited intertidal spat catching and holding leases and the increased demand for spat, hatchery spat has recently become available. Overseas markets desire large half-shell oysters, whereas the developing subtidal industry requires rapid growth rates to avoid fouling organisms and spionid infestations (M. Hippolite, pers. comm.).

The aims of this study were to optimize oyster production methods by: (1) further determining the seasonal timing of spionid polychaete infestations and investigating infestations, oyster condition, and growth over two depth regimens; and (2) investigating the effects of stunting spat in the intertidal for varying periods on oyster growth rates and condition and trying an alternative spat-holding technique for hatchery seed by stunting spat in a recirculating tank limited in food supply.

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Figure 1. Oyster farm locations and spat collecting site in the Marlborough Sounds, New Zealand.

METHODS

Spionid Infestations

Oyster spat were collected on polyvinyl chloride (PVC) sticks (Wyborn 1986) during February to March 1993 at an intertidal lease in Croisilles Harbour (Fig. 1). Spat sticks were suspended from the water surface in early December 1993 at two sites in Admiralty Bay, Marlborough Sounds (Fig. 1), using longline techniques developed for the mussel industry (Hippolite 1993). These sites were adjacent to the farms used in a preceding study of the occurrence of spionid polychaete infestations in cultivated oysters (Handley 1995). Three replicate "droppers" consisting of five 1.2-m tubular sticks threaded vertically on rope were suspended over two depth ranges. The surface droppers were suspended from 0.5 to 6.5 m, and the deep water droppers were suspended from 6- to 12-m depth. Site "A" was 31 m deep, and site "B" was 25 m deep.

The clumped growth characteristics of the oysters on the sticks prevented true random sampling (Eberhardt 1976), so each month, 20 oysters were haphazardly sampled from each replicate dropper at the two depth regimens. Salinity and temperature were recorded at 1-, 5-, 10-, and 15-m depth at both sites during sampling, and after they were scraped with a knife and scrubbed clean of fouling organisms, 10 of the oysters were placed overnight in a seawater solution of 0.5% phenol and 0.25% di-chlorobenzene (MacKenzie and Shearer 1959) to extract any spionid polychaetes from the shells. Extracted polychaetes were fixed in 10% borax-buffered formalin in seawater and identified to species level (Hartman 1943, Rainer 1973, Read 1975). The remaining 10 oysters were frozen for later dry weight condition index (DWCI) derivation after drying the meat and shells to a constant weight at 60°C; $DWCI = \text{dry meat weight (g)/dry shell weight (g)} \times 1,000$ (Roper et al. 1990). The greatest dimension (height) of the dried oyster shells was recorded with callipers to the nearest millimeter. All 20 oysters from each sample were graded on their degree of shell blistering (Table 1). Shells with a grade of 2 or more were considered unsuitable for the half-shell market.

The experiment was designed to run for 12 mo, but because the oysters remained relatively free of spionid infestations during this

TABLE 1.

Description of the visual grading index for Pacific Oyster shells.

Grade 0	No evidence of mudblisters or boring visible on the inside of the shells.
Grade 1	$\leq 25\%$ of the internal shell surface area as mudblisters, or any shells containing evidence of boring.
Grade 2	$>25, \leq 50\%$ of the internal shell surface area as mudblisters, or any oyster containing "larval new blisters" (see Results).
Grade 3	$>50, \leq 75\%$ of the internal shell surface area as mudblisters.
Grade 4	$>75\%$ of the internal shell surface area as mudblisters.

period, the experiment was monitored for a further 3 mo until all of the oysters had been removed from the sticks. The resulting incomplete data sets prevented full statistical analysis of the results. Data were pooled across replicates and displayed with 95% confidence intervals, which is the most clear and meaningful presentation of the statistical analysis (Jones and Matloff 1986).

Spat Stunting

Oyster spat were caught on tubular PVC sticks at the same spat catching site as above during March 1993 (approx. 12–15 dozen/stick; Fig. 1). In May 1994, half of the experimental oyster sticks (48 sticks) were transferred to a shellfish hatchery 12 km from Nelson. Spat were held submerged in a recirculating tank of approximately 5,000-L total volume: "submerged spat." The remaining spat were left to harden in the intertidal at extreme low water neap: "hardened spat." Submerged spat were dipped at monthly intervals in freshwater to control fouling organisms and spionid worms. The submerged spat were supplied with unfiltered seawater from Tasman Bay at monthly intervals and were thus assumed to be food limited. At approximate monthly intervals from June 1994 to January 1995, three oyster sticks per spat treatment were hung out vertically on longlines at two marine farm leases—one on site B in Admiralty Bay and the other in Wairangi Bay, Croisilles Harbour (Fig. 1). The oysters at site "B" in Admiralty Bay were attached to the northern end of a stocked mussel longline, and oysters in Wairangi Bay were grown on commercial oyster longlines.

Estimates of growth were derived by measuring 20 haphazardly selected oysters per treatment, per farm, each month to the nearest millimeter using calipers. When the majority of oysters had reached a minimum harvestable size (>70 mm), two replicate samples each of 20 oysters were removed from each stunting treatment within each monthly treatment. After the oysters had been cleaned, 10 oysters were frozen for DWCI evaluation, all 20 oysters were graded on the degree of shell blistering, and their height dimensions were recorded as above.

All data were checked for normality and homogeneity of variance before analyses of variance (ANOVA) were performed to satisfy the assumptions of the tests. Data were \log_{10} transformed before statistical analysis as they were related to growth (LaBarbera 1989). Four-way ANOVA were performed using the statistical program SAS (PROC GLM, SAS Institute Inc. 1992) to compare means of oyster shell grades, dry meat and shell weights, condition index values, and shell heights in order to test for differences between sites, months, spat treatments, and replicates. These data were first analyzed by mixed-model ANOVA using the

means' square error for the random factor "replicate" to test for significance (Underwood 1981). If the variation among replicates proved insignificant, the models were reanalyzed using fixed-factor ANOVA (B. McArdle, pers. comm.). Data were pooled across replicates and displayed with 95% confidence intervals.

RESULTS

Spionid Infestations

Spionid Species and Frequencies

Four spionid species of the genus *Boccardia* and three species of the genus *Polydora* were extracted from oysters grown in Admiralty Bay during the austral summer of 1994–1995 (Table 2). *B. knoxi* infestations increased substantially in this second summer to a maximum mean frequency of 13.3 worms extracted from three replicates of 10 oysters (± 6.5 95% confidence interval).

Shell Grades

Consistent with the spionid frequencies, the proportion of blistered oysters and the shell grades were very low for the first 12 mo of monitoring, with less than 10% of the oysters containing shell blisters at both farms and depths (Fig. 2A). Shell blistering rapidly increased from December 1994 to a maximum of 57% in March 1995 at the surface of site "B," with 17% of the final product unsuitable for the half-shell trade. The percentage of blistered oysters was greatest at 1- to 6-m depth at site "B" in January 1995 (Fig. 2A); however, mean shell grades were not significantly different between depths (Fig. 2B).

Oyster Condition

The condition of the oysters varied seasonally over the duration of this experiment (Fig. 3A). Oysters reached their greatest condition during late October 1994, after which their condition declined markedly. Separate analysis of the condition index parameters indicated that the shell weights increased with little variation through time (Fig. 3B), whereas the dry meat weights varied seasonally (Fig. 3C).

Oyster Growth

Oysters grew rapidly for the first 6 mo into midwinter (June 1994), especially those oysters at the surface of both sites, which grew significantly faster at the 95% confidence level than oysters at 6- to 12-m depth (Fig. 3D). Oyster growth rates decreased after June 1994, and the differences between depths became insignificant, except for the oysters at the surface of site "B," which grew very rapidly during October 1994.

Water Temperatures and Salinity

Water temperature was greatest at the end of summer in March 1994 and February 1995 (Fig. 4). Site "A" had a relatively constant temperature and salinity regimen with depth, suggesting that the water column was well mixed, whereas site "B" exhibited stratification with depth during the spring months where the temperature and salinity profiles appeared to be affected by freshwater runoff from a nearby stream.

Spat Stunting

Growth

Wairangi Bay oysters grew significantly larger than those in Admiralty Bay (Fig. 5). There were no significant differences in growth rates between hardened and submerged spat up to October 1994, but with regard to the oysters hung out from November, the hardened spat grew significantly larger than submerged spat at both farm sites (the January hardened batch in Admiralty Bay was lost before the last sampling date). At harvest, all oysters were similar in size within farm sites except the oysters hung out in January in Wairangi Bay, which were significantly smaller (Fig. 5). ANOVA detected significant differences between spat stunting methods and duration within sites (Table 3). The growth rates of the oysters were estimated from the difference between the mean first and final growth measurements; the true growth rates could not be measured because the populations sampled each month were not tagged individuals. The daily and monthly growth rates increased steadily each month, with the oysters hung out in January 1995 growing at almost twice the rate of those hung out in May 1994 (Table 4).

Condition

Oyster condition was significantly greater in Wairangi Bay than in Admiralty Bay (Fig. 6). Significant differences were detected for condition index values among months within sites; however, spat stunting treatment had no significant effect (Table 3). In Admiralty Bay, the oysters hung out in June 1994, especially the submerged spat, produced significantly heavier meat and shells compared with those produced in other months, but this was not shown by the condition index (Fig. 6, Table 3). In Wairangi Bay, however, meat and shell weights were similar for all oysters hung out before January, including the submerged spat from November, which were significantly smaller.

DISCUSSION

A spring infestation by *B. knoxi* was reported in a previous study of oysters grown in Admiralty Bay (Handley 1995). This phenomenon is further supported by the findings of this study. The intensity of infestation was similar in both studies, with the maximum infestation recorded at 59%. Oysters cultured in the vicinity of the previous sites had few infesting spionids or shell blisters during the first 12 mo of growth in suspended culture. Presumably, these oysters missed the settlement phase of *B. knoxi* in the spring of 1993, but were infested the following spring of 1994. Alternatively, the juvenile oysters could have been less prone to infestations than adults. The lag period observed by Handley (1995) was again evident between the increase in numbers of *B. knoxi* extracted from oysters (November to January 1994) and increased blister formation (January 1995).

Read (1975) observed *B. knoxi* to have two larval forms. The first is a nonplanktonic form using adelphophagia for direct development, which is produced throughout the year. The second is planktonic and much less common releasing larvae in the spring (September to October; Read 1975, pers. comm.). Observations of infestation patterns from this and the previous study (Handley 1995) are consistent with a spring infestation period by planktonic larvae of *B. knoxi*, resulting in shell blisters during November to February. An alternative hypothesis of adelphophagic larvae being carried up into the water column by turbulent processes (Cumings et al. 1995) and contributing to infestations seems unlikely.

TABLE 2.

Frequency of spionid polychaete species extracted from three replicate droppers of 10 oysters at two depths and two farms in Admiralty Bay.

Parameter			<i>Boccardia</i>												<i>Polydora</i>								
			<i>B. otakunika</i>			<i>B. chilensis</i>			<i>B. knoxi</i>			<i>B. magnioyda</i>			<i>P. armata</i>			<i>P. dorsomaculata</i>			<i>P. websteri</i>		
Date	Farm	Depth	Tot.	X	CI	Tot.	X	CI	Tot.	X	CI	Tot.	X	CI	Tot.	X	CI	Tot.	X	CI	Tot.	X	CI
April 27, 1994	A	S							3	1.0	1.1				1	0.3	0.7				2	0.7	0.7
		6							1	0.3	0.7				3	1.0	0.7						
	B	S							3	1.0	1.1										1	0.3	0.7
		6													1	0.3	0.7						
June 3, 1994	A	S				3	1.0	1.1										1	0.3	0.7	1	0.3	0.7
		6	2	0.7	1.3																		
	B	S				1	0.3	0.7															
		6							1	0.3	0.7												
July 11, 1994	A	S										1	0.3	0.7									
		6				1	0.3	0.7															
	B	S																					
		6				4	1.3	1.7				1	0.3	0.7									
August 11, 1994	A	S							1	0.3	0.7										1	0.3	0.7
		6																					
	B	S													3	1.0	1.1						
		6																					
September 21, 1994	A	S							1	0.3	0.7												
		6							1	0.3	0.7												
	B	S							1	0.3	0.7				1	0.3	0.7				1	0.3	0.7
		6							1	0.3	0.7										2	0.7	1.3
October 25, 1994	A	S				1	0.3	0.7	4	1.3	2.6				3	1.0	2.0				2	0.7	1.3
		6							1	0.3	0.7										1	0.3	0.7
	B	S				1	0.3	0.7	5	1.7	2.4												
		6																					
November 30, 1994	A	S							3	1.0	2.0										1	0.3	0.7
		6							1	0.3	0.7												
	B	S							4	1.3	0.7												
		6							3	1.0	1.1												
January 15, 1995	B	S							38	12.7	8.0												
		6							15	5.0	3.0												
March 1, 1995	B	S							40	13.3	6.5										2	0.7	0.7

Note: no spionids were present before April 27, 1994. Tot., total; X, mean; CI, 95% confidence interval; S, surface, 6, 6-m depth.

given the depth of water at the two sites in Admiralty Bay (>10 m from substratum to the oysters). *B. knoxi* also reproduces throughout the year in Wellington Harbour (Read 1975), yet infestations have only been observed during spring in the Marlborough Sounds.

Results of this study indicate that the spring infestation period of *B. knoxi* could be avoided by hanging out oyster spat during

December and harvesting them before December the following year, before shell blisters are induced by subsequent infestations. This growth period would, however, impose restrictions on the size of oysters produced free of blisters (estimated size, 70–80 mm). Alternatively, oysters could be grown through the blister induction period until the blisters have been covered with adequate shell material to be acceptable to the market. However, this would

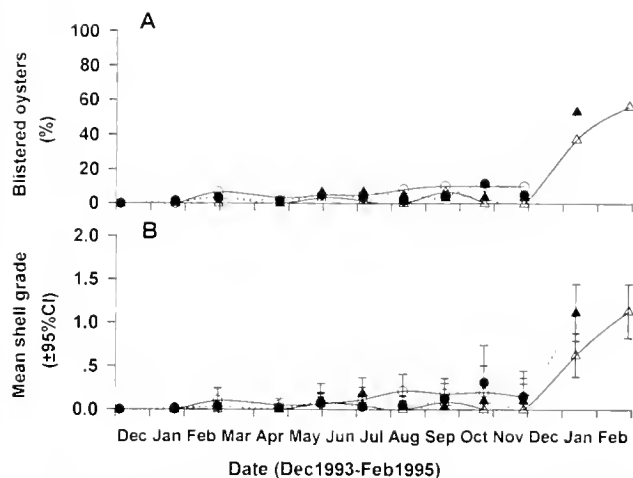


Figure 2. (A) Percentage of oysters containing shell blisters. (B) Mean shell grades of oysters grown in Admiralty Bay from December 1993 to February 1995 (three replicates of 20). (○) Site "A," surface; (●) Site "A," 6 m; (△) Site "B," surface; (▲) Site "B," 6 m; CI, confidence interval.

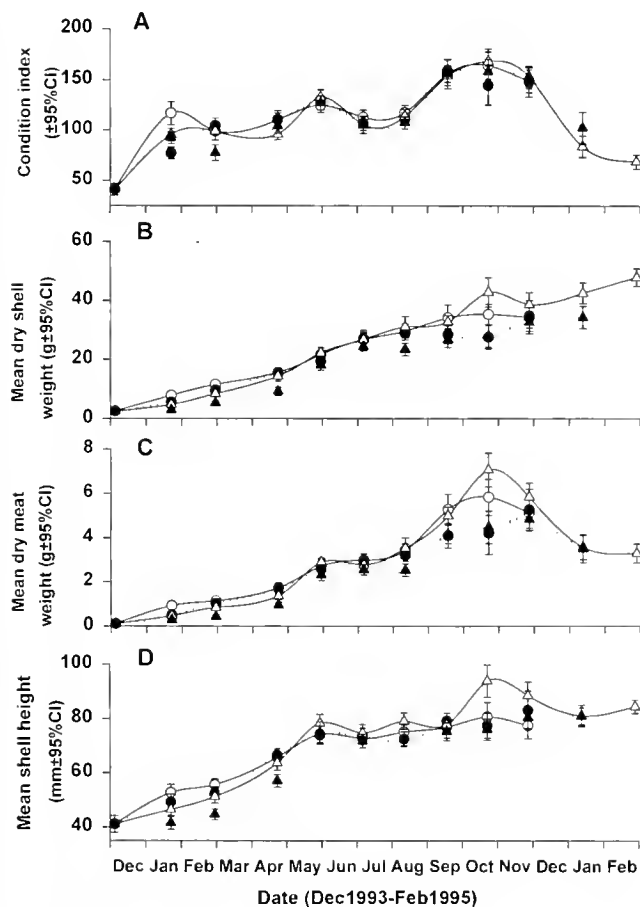


Figure 3. (A) Mean condition index values (three replicates of 10); (B) mean dry shell weights ($n = 30$); (C) mean dry meat weights (three replicates of 10); (D) mean shell height (three replicates of 20) for oysters grown in Admiralty Bay from December 1993 to February 1995. (○) Site "A," surface; (●) Site "A," 6 m; (△) Site "B," surface; (▲) Site "B," 6 m; CI, confidence interval.

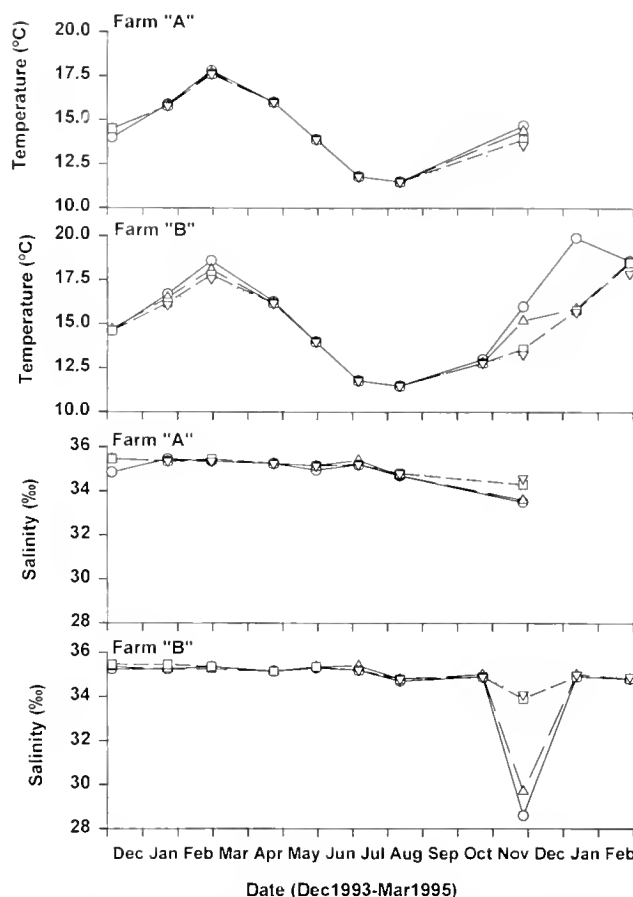


Figure 4. Temperature and salinity records for the two marine farm sites "A" and "B" in Admiralty Bay between December 1993 and March 1995; (○) 1 m, (△) 5 m, (□) 10 m, (▽) 15 m.

not be advisable because the oysters at site "B" rapidly lost condition through the loss of meat weight, presumably due to spawning in late November. Thus, an annual crop rotation strategy is recommended, allowing optimization of longline use and meat condition and avoiding shell blistering.

The percentage of oysters containing shell blisters during January 1994 at site "B" indicated that shell blistering was greater below 6 m of depth. This difference appeared to be the result of increased shell growth, indicated by the greater dry shell weights (Fig. 3), rather than differential levels of *B. knoxi* infestation, which did not significantly differ between depths (Table 2). Perhaps higher surface temperatures at this site led to increased oyster growth (Brown and Hartwick 1988). Korringa (1952) noted that *Polydora* infestations of vigorous oysters produced minimal effects because the worms were only in contact with the mantle for a very short period before being isolated by blister formations. Further understanding of the relationships between oyster condition factors and controlling environmental parameters such as temperature, salinity, and food resources, as well as shell deposition strategies used to combat spionid infestations, would help maximize oyster production in areas of high spionid infestations.

The increased growth rates resulting from longer stunting periods observed in Admiralty Bay and Wairangi Bay could not be exclusively attributed to seasonal changes in water temperature or increasing food availability toward the austral summer (December to January) because all of the oysters except those hardened until

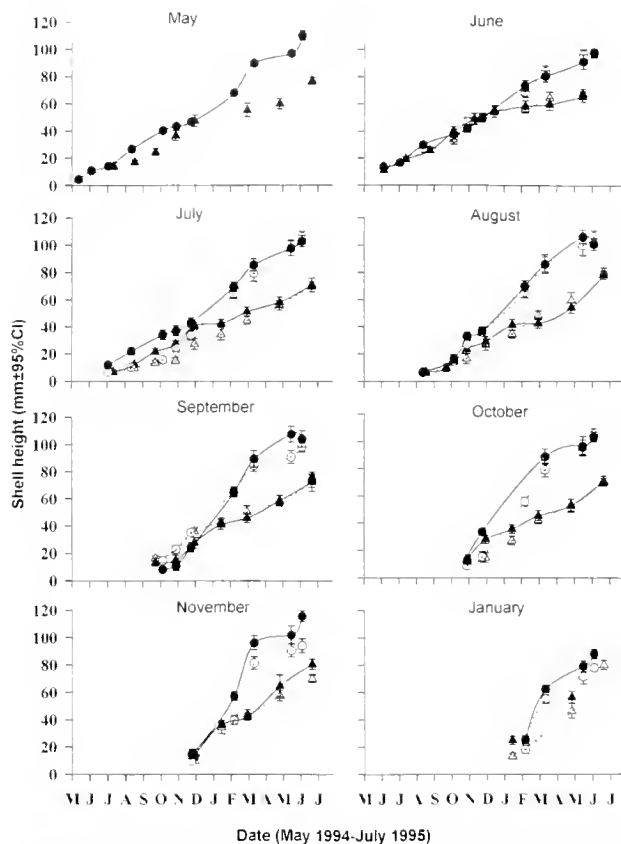


Figure 5. Oyster growth measured by greatest shell height at the two oyster farm sites; Admiralty Bay site "B": (▲) hardened spat, (△) submerged spat; Wairangi Bay: (●) hardened spat, (○) submerged spat. Spat were hung out from May 1994 to January 1995.

January 1995 reached a similar size range at the completion of the experiment. Thus, the hardening or stunting period increased the growth potential of the oysters up to an optimal point in November (approx. 8 mo), after which the growth rates were not sufficient to allow the oysters to reach an equivalent size at the time of harvest. In terms of oyster production, this increased growth rate is very desirable because it allows the farmer to stunt spat in order to avoid mudworm infestations and fouling organisms, which are the major cause of loss of production and value of subtidal oysters in

the Marlborough Sounds. During the summers of 1993 (Handley 1995) and 1995 (above) up to 17% of subtidal oysters monitored in Admiralty Bay were rendered unsuitable for the lucrative half-shell trade because of infestations of *B. knoxi*. The most common fouling organisms of subtidal oyster cultures in the Marlborough Sounds are the blue mussels, *Mytilus edulis*, tube worms (*Pomatoceros* sp.), barnacles (*Balanus* sp.), filamentous bryozoans, and compound ascidians (pers. observ). The extent of the fouling is, however, highly variable between sites and between years. If oysters become too fouled, the increased weight of the fouling organisms can strip the oysters off of their settlement surface as they reach a harvestable size. Alternatively, the added weight can contribute to structural failures, breaking ropes, or sinking longlines (R. Hippolite, pers. comm.). It is therefore desirable to reduce the growout period to minimize oversettlement by fouling organisms and to avoid spionid infestations.

Research by Ogasawara et al. (1962) leading to the current practice of intertidal spat hardening has shown that *C. gigas* spat that survive settlement in the intertidal have a greater adaptability to varying environmental conditions and put less effort into reproduction. Subsequent reduced water content of oysters that survived the hardening process was correlated with an increased recovery after spawning and greater survival, with few "watery oysters" produced. Intertidal bivalves have evolved energy-conserving and energy-supplementing adaptations to minimize the limitations placed on growth by intertidal conditions, and some species may have higher growth rates at certain intertidal levels (Gillmor 1982). Some intertidal bivalves are capable of digestive activity during the intertidal exposure period, and it has been suggested that intertidal exposure of some bivalve species may facilitate coordination of digestive activity (Gillmor 1982). This has been shown by increased growth of *C. gigas* spat when fed discontinuously (Langton and McKay 1974, Langton and McKay 1976). The progressive increase in growth rates with stunting period in this study could have been explained by differential survival of faster growing spat. This seemed unlikely, however, because the densities of oysters on the sticks did not appear to change between the different stunting treatments or stunting methods. The results therefore support the findings of Crosby and Gale (1990), indicating that the energy-compensatory and energy-conserving mechanisms of intertidal bivalves can be cumulative. Further, because spat stunted by limiting food in the recirculating tank also exhibited these adaptive mecha-

TABLE 3.

ANOVA comparing variables among: Site, farm locations; Mon, month spat hung out; Tre, spat stunting treatment; n/rep, number of oysters per replicate.

Source	Variable: n/rep:	Shell Grade	Shell Height	Condition Index	Dry Meat Weight	Dry Shell Weight
		20	20	10	10	10
Site		**	***	***	***	***
Mon		***	***	***	***	***
Tre		ns	ns	ns	ns	ns
Site × Mon		***	***	**	***	***
Site × Tre		ns	***	*	ns	**
Mon × Tre		*	***	ns	***	***
Site × Mon × Tre		ns	***	***	***	***
Replicate (Site × Mon × Tre)		ns	ns	ns	ns	*

* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$; ns, not significant.

TABLE 4.

Monthly estimated growth rates of oysters derived from the mean first and final height measurements.

Oyster Growth Rates							
Location	Month	Spat	Growth/Month (mm)	Location	Month	Spat	Growth/Month (mm)
Admiralty Bay	May	H	4.8	Wairangi Bay	May	H	7.8
	Jun	H	5.3		Jun	H	6.5
		S	5.9			S	6.2
	Jul	H	4.8		Jul	H	7.0
		S	5.0			S	8.3
	Aug	H	6.6		Aug	H	8.9
		S	6.8			S	9.6
	Sept	H	6.1		Sept	H	10.8
		S	5.9			S	9.7
	Oct	H	6.4		Oct	H	11.8
		S	7.0			S	11.8
	Nov	H	7.1		Nov	H	14.6
		S	9.2			S	12.2
	Jan	H			Jan	H	14.3
		S	9.8			S	13.6

Oysters were hung out from May 1994 to January 1995. H, hardened spat; S, submerged spat.

nisms, it is proposed that reduced energy acquisition, rather than discontinuous feeding activities present in the intertidal, controls these mechanisms.

Neither the spat stunting technique nor its duration had a significant effect on the condition of oysters. Larger meat weights consistent with larger overall size were produced in Wairangi Bay with no significant variations between months. When the meat and shell weights were analyzed separately, the greatest meat weights were produced in Admiralty Bay by hanging out submerged spat in June; however, these oysters subsequently contained a higher percentage of shell blisters (47.5%; unpubl. data). In Wairangi Bay, the greatest meat weights were produced by hanging out spat in September to October. Taking oyster condition, growth rates, and spionid infestations into consideration, approximately 8 mo (October) was optimal for stunting spat in the recirculating tank, and approximately 9 mo (November) was optimal for spat held intertidally.

The intersite differences in growth rates and condition observed between Admiralty Bay and Wairangi Bay were most likely due to site-specific characteristics, including greater fresh water inputs and longer residence times, resulting in higher productivity with greater phytoplankton biomass in Wairangi Bay (L. MacKenzie and K. Todd, pers. comm.). Admiralty Bay is a relatively deep water mass compared with Wairangi Bay, receiving a mixture of waters from Tasman Bay (via French Pass; Fig. 1), Cook Strait, and outer Tasman Bay (Heath 1976, Heath 1985). Wairangi Bay, sheltered within Croisilles Harbour, is fed Tasman Bay water traveling in a mean anticlockwise direction in Tasman Bay (Heath 1976). The hydraulic residence time in Croisilles Harbour is expected to be relatively long, with parcels of water expected to move backward and forward within the harbor, rather than out into Tasman Bay (R. Roberts and S.J. Handley, unpubl. report). The Wairangi Bay site was also closer to a significant freshwater source than the Admiralty Bay site.

This study has shown that in Admiralty Bay, *B. knoxi* was the primary cause of *C. gigas* shell blistering after a spring infestation period. Stunting the growth of *C. gigas* spat during early devel-

opment had demonstrated effects on the duration of growout in the subtidal, which has advantages for commercial oyster growers. Managing the cumulative adaptive capabilities of oysters stunted either by limiting food supply in tanks or by reducing food availability in the intertidal can be used to optimize subtidal growth rates, thus reducing the growout time, avoiding fouling and spionid infestations, and thus maximizing the use of marine farm structures. To optimize oyster condition and growth and minimize spio-

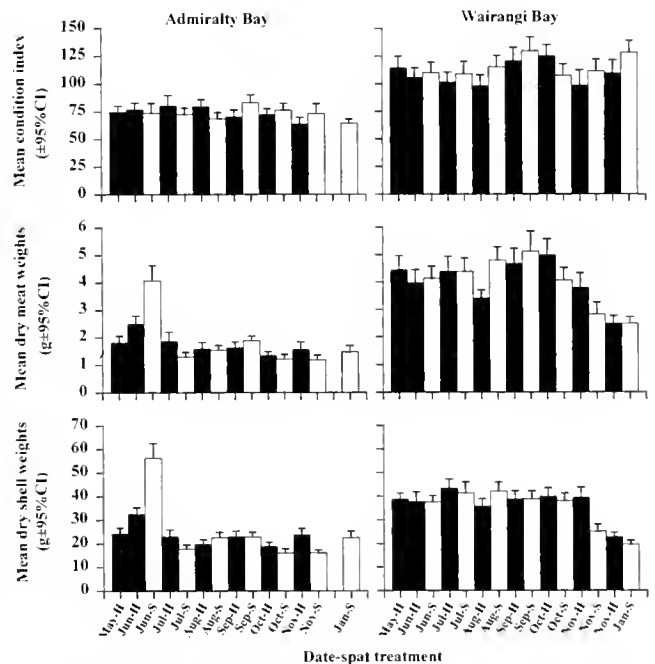


Figure 6. Mean oyster condition index, dry meat weights, and dry shell weights for oysters from Admiralty Bay site "B" (harvested June 23, 1995) and Wairangi Bay (harvested June 6, 1995). Spat were hung out from May 1994 to January 1995; black bars/H, hardened spat; clear bars/S, submerged spat.

nid-induced shell blistering, an annual crop rotation should be used in areas prone to *B. knoxi* infestations. In areas outside the range of *B. knoxi*, oyster spat caught intertidally in March should be stunted for 8 mo intertidally or 9 mo in a recirculating tank to maximize subtidal growth and thus decrease the growout period.

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GROWTH AND SURVIVAL OF *SPISULA SOLIDISSIMA SIMILIS* LARVAE FED DIFFERENT RATIONS OF TAHITIAN STRAIN *ISOCHRYDIS* SPECIES

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ABSTRACT Laboratory-spawned veliger-stage larvae of the southern Atlantic surfclam, *Spisula solidissima similis* (Say 1822), were reared to late pediveliger stage on five different cell concentrations of Tahitian strain *Isochrysis* species (T-Iso) to determine an optimal food ration for this subspecies. Larvae were fed daily 0, 50,000, 100,000, 200,000, or 300,000 cells/mL of T-Iso. Day-old veliger larvae were stocked in 150 (1-L) replicate flasks at mean densities of 0.7 or 0.8 larvae/mL for trials A and B, respectively. Larval growth and survival were assessed every 2 days over the 14-day trial periods. Significantly greater growth and survival of larvae occurred in both trials in the lower food rations of 50,000 and 100,000 cells/mL. A reduction in larval growth rate and survival was observed at the higher ration treatments. A decline in overall larval health may be associated with the deleterious effects of surplus ration degradation.

KEY WORDS: *Spisula*, larvae, aquaculture, food ration, growth, survival

INTRODUCTION

The economic importance of the Atlantic surfclam, *Spisula solidissima solidissima* (Dillwyn 1817), fishery has been recognized for decades (Ropes 1968). The surfclam fishery, the second leading clam fishery in terms of dollars earned in the United States, produced 73.9 million pounds of meat valued at 34 million dollars in 1993 (O'Bannon 1994). The southern subspecies, *Spisula solidissima similis* (Say 1822), which occurs from Massachusetts to Florida and through the Gulf of Mexico to Texas (Abbott 1974), is not commercially harvested. In contrast to *S. s. solidissima*, which has been extensively studied in terms of its natural history (Ropes 1968) and fishery (Merrill and Ropes 1969) and aquacultural potential (Goldberg 1980, Goldberg and Walker 1990, Walker and Heffernan 1991), far less economic or natural history information is available on *S. s. similis*.

Growth and longevity studies (Walker and Heffernan 1994) indicate that *S. s. similis* individuals from inshore populations in Georgia reach a mean maximum shell length of 47–48 mm, with a mean longevity of 1.5 y. Also, in Georgia, *S. s. similis* mature sexually by January to February and spawn from March until May (Kanti et al. 1993). Coupled, these studies would seem to indicate that *S. s. similis* has good aquacultural potential for the lucrative raw, fried, and steamer markets, as well as the pasta clam market.

Optimal salinity and temperature regimens for *S. s. similis* embryo-to-larval metamorphosis in a laboratory have also been documented (Walker et al. 1995). The effect of food ration quality and quantity is an important aspect of bivalve larval husbandry (Loosanoff et al. 1955, Pratt and Campbell 1956, Epifanio et al. 1976, Goldberg 1980, Rhodes et al. 1984) and in this case needs to be addressed on the subspecies level. Therefore, the objective of this study was the determination of the optimal feeding ration based on cell numbers for hatchery-reared *S. s. similis* larvae fed Tahitian strain *Isochrysis* species (T-Iso) through metamorphosis.

METHODS

Adult southern Atlantic surfclams, *S. s. similis*, were captured as broodstock from St. Catherine's Sound, GA, in early February 1993. After acquisition, clams ($n = 272$ total) were equally divided into two, 6-mm mesh, 70 × 70 × 20 cm, vinyl-coated wire cages placed below mean-low water, on a sandy-tidal flat at the mouth of House Creek in Wassaw Sound, GA. On March 8, the broodstock were returned to the laboratory and placed in 400-L conditioning tanks maintained at 13°C and 26 ppt salinity. The broodstock were randomly divided into three equal size groups ($n = 87$), and placed in separate conditioning tanks. Clams were acclimated to temperatures of 15, 20, and 25°C in preparation for another experiment (Walker and Hurley 1995), by increasing the water temperature by 1°C daily. Unsolicited mass spawning occurred on April 3 and 4 from broodstock held in the 15 and 20°C conditioning tanks, respectively. Each resulting larval cohort was held in separate tanks maintained at 20°C and allowed to develop into veligers before the commencement of the experimental trials.

On April 4, Feeding Trial A was initiated from the 1-day-old veliger larvae of the 15°C conditioned broodstock. Larvae were stocked in nonaerated, 1-L flasks ($n = 160$ total) at a concentration of 0.7 larvae/mL. Water salinity of 25 ppt and temperature of 20 ± 1°C were maintained constant throughout the trial for all larval treatments. Larval treatments consisted of a ration of T-Iso at concentrations of 0, 50,000, 100,000, 200,000, and 300,000 cells/mL delivered once daily. All replicate flasks received a water exchange and a thorough rinse through a 20-μm-pore-size sieve on alternate days. On each alternate day, immediately before the water exchange, the contents of each of four flasks per treatment were sieved through a 20-μm-pore-size screen, fixed in a 10% buffered Formalin and Rose Bengal stain solution (v:v) and concentrated to a 50-mL suspension. Initial stocking density validation was based on three replicate 1-mL counts for each of four flasks per treatment at Time 0. Survival estimates were based on Sedgewick-Rafter

slide counts of three 1-mL subsamples per replicate suspension per treatment per sample period. All survival data were proportionally adjusted to the original replicate flask culturing volume (1 L) before statistical analysis. Shell length measurements (maximum anterior-posterior distance) were taken for 30 animals per treatment per sample date (see Heffernan et al. 1991).

On April 5, Trial B was initiated with 1-day-old veliger larvae from the 20°C conditioned broodstock spawning. Larvae were stocked at a density of 0.8 larvae/mL in 1-L flasks ($n = 150$ total) with treatments consisting of a daily deliverance of 0, 50,000, 100,000, 200,000, and 300,000 cells/mL of T-Iso. Larval treatments in Trial B were fed, maintained, and sampled as described above for Trial A.

Differences in growth and survival among treatments were determined by analysis of variance ($\alpha = 0.05$) and Tukey's Studentized Range Test ($\alpha = 0.05$). All percent survival data were arcsine transformed before statistical analysis. Statistical analysis was performed on SAS for PC (SAS Institute Inc. 1989).

RESULTS

Statistically, greater stocking densities of veligers ($p = 0.0178$) occurred in the 200,000 cells/mL treatment at the initiation of the study; thus, the 200,000 cells/mL treatment was eliminated from Experimental Trial A. No significant differences in larval stocking size existed among the remaining treatments ($p = 0.1732$) at Time 0 (Table 1). On Day 2, the larvae from treatments given no food and 50,000 cells/mL were equal in size, but significantly smaller than the two higher density ration treatments ($p < 0.0001$). Days 4, 6, and 8 exhibited the same trends with significantly greater larval size in all fed treatments compared with the unfed treatment (all $p < 0.0001$). By Day 10, the unfed treatment had no surviving larvae and was discontinued. Also, by Day 10 ($p < 0.0001$) and on subsequent Days 12 ($p = 0.0005$) and 14 ($p = 0.0060$), larvae from the 50,000 and 100,000 cells/mL ration treatments were not significantly different from each other but were significantly larger than those from the 300,000 cells/mL treatment ($p < 0.0001$).

No significant differences in larval stocking densities existed among the remaining treatments of Trial A at Time 0 ($p = 0.1433$; Table 2). Significantly lower larval survival occurred on Day 2 for the 50,000 cells/mL treatment ($p < 0.0001$), whereas significantly lower survival occurred for both the unfed and 50,000 cells/mL treatments ($p < 0.0004$) on Day 4. No significant differences in survival occurred among fed treatments on Day 8 ($p = 0.2463$) or 10 ($p = 0.0871$). Significantly lower survival occurred in the 300,000 cells/mL treatment on both Days 12 ($p = 0.0017$) and 14 ($p = 0.0002$) as compared with the lower food ration treatments of 50,000 and 100,000 cells/mL.

In Trial B, statistically higher larval stocking densities occurred at Time 0 in the 300,000 cells/mL ration treatment ($p = 0.0283$), and this treatment was thus eliminated from the trial. No significant differences in larval size existed among the remaining treatments at Time 0 ($p = 0.4749$) or Day 2 ($p = 0.2251$) (Table 3). By Day 4 and on subsequent days, all fed treatments exhibited significantly larger larval size (all $p < 0.0001$) compared with the unfed treatment. On Days 6 and 10, larvae from the 50,000 and 100,000 cells/mL treatments were not significantly different in size; however, larvae in both treatments were significantly smaller than those in the 200,000 cells/mL treatment ($p < 0.0001$ and $p = 0.0081$, respectively). Additionally, the unfed treatment had no surviving larvae by Day 10 and was discontinued. On Days 12 and 14, larvae in the 50,000 and 100,000 cells/mL treatments were significantly larger than the 200,000 cells/mL treatment larvae ($p < 0.0001$ for both days).

No significant differences in larval density existed between treatments in Trial B at Time 0 or Day 2 ($p = 0.9811$ and $p = 0.1757$, respectively) (Table 4). On Day 4, no significant differences in larval survival existed between the 50,000 cells/mL and the unfed treatments; however, both treatments had significantly lower survival than the higher ration treatments of 100,000 and 200,000 cells/mL ($p < 0.0001$). On Days 6 ($p = 0.7504$) and 8 ($p = 0.4924$), no significant differences in larval survival existed among treatments. By Day 10, the unfed treatment exhibited total

TABLE 1.
Trial A mean size (μm) of *S. s. similis* larvae ($\pm\text{SE}$) fed four different food rations (cells/mL) of T-Iso.

Sample Day and Tukey's Ranking	Treatment (Cells/mL per Day)			
	300,000	100,000	50,000	0
Day 0 ($p = 0.1732$)	73.6 \pm 0.4(a)*	73.4 \pm 0.3(a)	74.3 \pm 0.4(a)	73.3 \pm 0.3(a)
Day 2 ($p < 0.0001$)	83.3 \pm 0.7(a)	81.4 \pm 0.4(a)	75.5 \pm 3.3(b)	78.3 \pm 0.2(b)
Day 4 ($p < 0.0001$)	91.4 \pm 0.9(a)	90.1 \pm 0.8(a)	88.8 \pm 0.9(a)	78.6 \pm 0.3(b)
Day 6 ($p < 0.0001$)	112.9 \pm 1.5(a)	108.2 \pm 1.7(a)	108.7 \pm 1.8(a)	81.2 \pm 0.6(b)
Day 8 ($p < 0.0001$)	130.0 \pm 2.1(a)	133.3 \pm 2.4(a)	131.5 \pm 2.3(a)	84.0 \pm 0.7(b)
Day 10 ($p < 0.0001$)	136.1 \pm 2.5(b)	161.0 \pm 3.6(a)	151.9 \pm 4.4(a)	†
Day 12 ($p = 0.0005$)	147.9 \pm 4.0(b)	179.6 \pm 4.9(a)	191.4 \pm 7.1(a)	†
Day 14 ($p = 0.0060$)	125.0 \pm 20.9(b)	193.4 \pm 8.0(a)	172.3 \pm 6.7(a)	†

* Letters in parentheses adjacent to mean larval size identify the Tukey's ranking.

† Total mortality observed.

TABLE 2.

Trial A percent mean survival (\pm SE) (larvae/mL) of *S. s. similis* larvae fed four different food rations (cells/mL) of T-Iso.

Sample Day and Tukey's Ranking	Treatment (Cells/mL per Day)			
	300,000	100,000	50,000	0
Day 0				
(p = 0.1433)	0.741 \pm .038(a)*	0.600 \pm .084(a)	0.562 \pm .060(a)	0.642 \pm .073(a)
Day 2				
(p < 0.0001)	0.558 \pm .046(a)	0.518 \pm .048(a)	0.050 \pm .014(b)	0.579 \pm .026(a)
Day 4				
(p = 0.0004)	0.863 \pm .051(a)	0.879 \pm .095(a)	0.463 \pm .079(b)	0.513 \pm .0963(b)
Day 6				
(p = 0.0037)	0.704 \pm .070(a)	0.738 \pm .070(a)	0.792 \pm .062(a)	0.463 \pm .050(b)
Day 8				
(p = 0.2463)	0.679 \pm .046(a)	0.692 \pm .053(a)	0.613 \pm .083(a)	0.521 \pm .073(a)
Day 10				
(p = 0.0871)	0.479 \pm .057(a)	0.575 \pm .032(a)	0.446 \pm .030(a)	†
Day 12				
(p = 0.0017)	0.142 \pm .015(b)	0.433 \pm .044(a)	0.500 \pm .093(a)	†
Day 14				
(p = 0.0002)	0.042 \pm .088(b)	0.283 \pm .046(a)	0.366 \pm .056(a)	†

* Letters in parentheses adjacent to larval count identify Tukey's rankings.

† Total mortality observed.

mortality and was discontinued. On Days 10 (p = 0.1117), 12 (p = 0.3673), and 14 (p = 0.0837), no significant differences in larval survival existed among the remaining treatments.

DISCUSSION

Food ration is an important consideration of bivalve larval culture and has been demonstrated to affect larval survival and growth in cultured *Mercenaria mercenaria* (Loosanoff and Davis 1963, Castagna and Krauter 1981, Riisgard 1988), *Ostrea edulis* (Helm and Laing 1987), *Crassostrea gigas* (Nascimento 1980), *Mytilus*

edulis (Riisgard 1991), and *S. s. solidissima* (Goldberg 1985). In our study, *S. s. similis* larvae had significantly greater growth and survival at food rations of 50,000 and 100,000 cells of T-Iso/mL than at higher cell concentrations. Consequently, these food ration treatments are interpreted by us as the optimal treatments, among those tested, for larval culture of *S. s. similis*. The observed developmental time of *S. s. similis* larvae fed the flagellate T-Iso to late pediveliger stage in this study (14 days) also approximates that of *S. s. solidissima* reared under similar temperatures and salinities, given a mixed daily ration of 100,000 cells/mL of *Pavlovi lutheri* and *Isochrysis galbana* (Goldberg 1980).

TABLE 3.

Trial B mean size (μ m) of *S. s. similis* larvae (\pm SE) fed four different food rations (cells/mL) of T-Iso.

Sample Day and Tukey's Ranking	Treatment (Cells/mL per Day)			
	200,000	100,000	50,000	0
Day 0				
(p = 0.4749)	73.9 \pm 0.2(a)*	73.6 \pm 0.3(a)	73.5 \pm 0.3(a)	73.3 \pm 0.2(a)
Day 2				
(p = 0.2251)	78.2 \pm 0.4(a)	77.4 \pm 0.3(a)	77.4 \pm 0.3(a)	77.6 \pm 0.3(a)
Day 4				
(p < 0.0001)	87.6 \pm 0.7(a)	90.4 \pm 0.8(a)	83.6 \pm 0.6(a)	82.0 \pm 0.5(b)
Day 6				
(p < 0.0001)	122.1 \pm 1.6(a)	113.7 \pm 1.6(b)	115.7 \pm 1.3(b)	87.1 \pm 0.6(c)
Day 8				
(p < 0.0001)	138.0 \pm 1.8(a)	138.4 \pm 2.0(a)	132.5 \pm 1.8(a)	95.5 \pm 0.8(b)
Day 10				
(p = 0.0081)	174.0 \pm 3.0(a)	161.1 \pm 2.8(b)	163.2 \pm 3.0(b)	†
Day 12				
(p < 0.0001)	179.4 \pm 3.6(b)	212.0 \pm 4.1(a)	218.7 \pm 4.3(a)	†
Day 14				
(p < 0.0001)	157.3 \pm 5.9(b)	224.3 \pm 4.2(a)	209.4 \pm 5.8(a)	†

* Letters in parentheses adjacent to mean larval size identify the Tukey's ranking.

† Total mortality observed.

TABLE 4.

Trial B percent mean survival (\pm SE) (larvae/mL) of *S. s. similis* fed four different food rations of T-Iso.

Sample Day and Tukey's Ranking	Treatment (Cells/mL per Day)			
	200,000	100,000	50,000	0
Day 0				
(p = 0.9811)	0.896 \pm .042(a)*	0.892 \pm .029(a)	0.900 \pm .068(a)	0.883 \pm .032(a)
Day 2				
(p = 0.1757)	0.988 \pm .058(a)	0.829 \pm .048(a)	0.846 \pm .077(a)	0.921 \pm .033(a)
Day 4				
(p < 0.0001)	0.758 \pm .094(a)	0.771 \pm .119(a)	0.183 \pm .067(b)	0.325 \pm .074(b)
Day 6				
(p = 0.7504)	0.770 \pm .061(a)	0.839 \pm .031(a)	0.796 \pm .055(a)	0.780 \pm .029(a)
Day 8				
(p = 0.4924)	0.750 \pm .036(a)	0.621 \pm .102(a)	0.729 \pm .058(a)	0.713 \pm .061(a)
Day 10				
(p = 0.1117)	0.829 \pm .046(a)	0.700 \pm .041(a)	0.792 \pm .036(a)	†
Day 12				
(p = 0.3673)	0.634 \pm .025(a)	0.733 \pm .021(a)	0.733 \pm .091(a)	†
Day 14				
(p = 0.837)	0.333 \pm .085(a)	0.625 \pm .119(a)	0.550 \pm .045(a)	†

* Letters in parentheses adjacent to larval count identify the Tukey's rankings.

† Total mortality observed.

Similar results were obtained in other laboratory studies using comparable cell concentrations with *M. mercenaria* larvae (40,000–60,000 cells/mL) fed T-Iso (Riisgard 1988) and *M. edulis* larvae (40,000–50,000 cells/mL) fed *I. galbana* (Jespersen and Olsen 1982). However, optimal bivalve growth rates in laboratory conditions may vary considerably from maximal growth rates in nature (Kiorhoe et al. 1981; Jorgensen 1990). It is presumed that these growth rate differences may be attributed to the adaptive responses of bivalves to laboratory conditions and the difficulty in creating optimal conditions such as feeding regimes and algal concentrations to which a bivalve species is adapted in the wild (Riisgard 1991). In veligers of both *M. mercenaria* (Riisgard 1988) and *M. edulis* (Jespersen and Olsen 1982), a reduction in the filtration rate due to higher algal concentrations was associated with reaching maximum gut retention, leading to valve closure, reduced metabolism, and reduced biosyntheses/growth (Riisgard 1991). Conversely, Perez-Camacho et al. (1994) found a direct relationship between ration cell numbers and larval clearance, ingestion, and growth in *Ruditapes decussatus* fed up to 300,000 cells/mL for short-time exposure (hours). The maricultural application of these results, however, may be misleading, because factors associated with prolonged exposure to higher cell densities could negatively affect larval survivability, as demonstrated in this study.

The consistency of the results for both larval size (Tables 1 and 3) and survival (Tables 2 and 4) further validates the finding that rations greater than 200,000 cells/mL are excessive and yield inferior larval production. Consideration of broodstock conditioning temperature is important in interpreting these results. Broodstock, embryo, and subsequent larval cohorts were subjected to different pre-experimental temperature treatments of 15 and 20°C (Trials A and B, respectively). Higher larval survival (33–63%; Table 4) occurred in Trial B, in which larvae were spawned in 20°C conditioning tanks and were reared at 20°C. Lower larval survival (4–37%; Table 2) occurred in Trial A, in which animals were conditioned in 15°C tanks but reared at 20°C. Thermal conditioning temperatures of broodstock and its effect on subsequent larval cohorts, therefore, may have confounded the results between the

optimal ration treatments; however, it played an apparently minor role in elucidating an optimal versus a suboptimal ration. Both trials demonstrated optimal larval growth and survival at food rations of 50,000 and 100,000 cells/mL. Previous work of broodstock conditioning effects on *S. s. similis* larvae (Walker and Hurley 1995) noted similar results, with 15°C conditioned broodstock yielding larvae of equal size and higher survivability as compared with larvae from 20°C conditioned broodstock. It is important to note that Walker and Hurley (1995) found the optimal conditioning temperature for *S. s. similis* broodstock to be 25°C, based on larval survival and size at 48 h.

Survival data (Tables 2 and 4) displayed no consistent trends among treatments until Days 10 (Trial A) and 12 (Trial B). Survival for Days 2 through 8 gave ambiguous results, fluctuating among the treatments and sample periods. By Days 10, 12, and 14, however, the lower food ration treatments of 50,000 and 100,000 cells/mL in both Trials A and B displayed greater survival than the higher ration treatments of 200,000 cells/mL (Trial B), or 300,000 cells/mL (Trial A). These increases suggest that ration densities approaching or exceeding 200,000 cells/mL are excessive.

The increased mortality and lower growth rates in the higher ration density treatments could be explained in part by an increase in deleterious microbial activity and contamination in the culture chambers (Loosanoff and Davis 1963). The decomposition of non-utilized algal cells promotes microbial activity, which can directly affect larval survival via exposure to harmful bacterial and fungal pathogens (Loosanoff and Davis 1963). Additionally, an increase in larval size results in an increase in larval oxygen demand (Riisgard et al. 1981). Prolonged hypoxia has been demonstrated to reduce both larval development and growth in *M. mercenaria* (Morrison 1971) and *M. edulis* (Wang and Widdows 1991). Excess food decomposition and increases in secondary production, coupled with an increase in oxygen demand as the larvae grow, could result in an overall decrease in available dissolved oxygen in the culture chambers. Suboptimal oxygen content in the rearing chambers would have created additional stress on the larvae. This oxygen stress coupled with contamination effects could reduce

larval vigor, which in turn could affect larval foraging ability and size. Metamorphosis is accountable for the primary loss of lipid reserves in developing larvae (Waldock and Holland 1978). The lack of an adequate accumulation of nutritional reserves necessary for the successful completion of "set" is frequently manifested as mortality associated with the final metamorphic stage (Loosanoff and Davis 1963). The lower growth rate of larvae in the higher ration treatments could be explained by an increase in the mortality of the larger, metamorphically competent individuals during this latter, physiologically stressful stage of their development.

Two primary culturing applications are evident from the results of this study. First, the cost benefits of reducing food ration cell density to approximately 75,000 cells/mL, as opposed to 150,000 cells/mL or greater, are significant. The algal production of T-Iso could be reduced by half, resulting in a concomitant reduction in necessary labor and expense. Second, both larval survival and

growth benefits associated with appropriate ration treatments will result in higher yields to the aquaculturalist.

In conclusion, this study has determined that the optimal food ration for developing *S. s. similis* larvae fed T-Iso is between 50,000 and 100,000 cells/mL in stocking densities of 0.7–0.8 larvae/mL. Increases in the food ration beyond 200,000 cells/mL result in both increased mortality and reduced growth rates.

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CULTURE OF SURFCLAMS *SPISULA SOLIDISSIMA* SP., IN COASTAL GEORGIA: NURSERY CULTURE

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ABSTRACT Growth of the Atlantic surfclam, *Spisula solidissima solidissima*, was compared with that of the southern Atlantic surfclam, *Spisula solidissima similis*. All experimental animals were reared in upweller units at 20 °C and fed cultured algae on a daily basis. Over the 14 wk of the study, the Atlantic surfclams grew markedly better (8.9-mm increase in shell length and a 1,103% increase in biomass) than the southern Atlantic surfclams (6.6-mm increase in shell length and 573% increase in biomass). Mortality for both groups was negligible. The mean shell lengths attained for the Atlantic surfclams (15.3 mm) and the southern Atlantic surfclams (13 mm) at the conclusion of the study were large enough to ensure good growth and survival on relocation to a field growout environment. The growth patterns obtained under similar growth conditions further highlight some basic life history differences between these subspecies, which were apparent from other studies.

KEY WORDS: *Spisula solidissima* sp., surfclam, growth, biomass

INTRODUCTION

The Atlantic surfclam, *Spisula solidissima solidissima*, has shown exceptional potential as a candidate for aquaculture in coastal Georgia (Goldberg and Walker 1990, Walker and Heffernan 1990, Walker and Heffernan 1990b). It has demonstrated excellent growth rates, with the ability to achieve commercial size within 1 y postfertilization (Goldberg and Walker 1990), while also achieving sexual maturity in 1 y (Spruck et al. 1995). The southern Atlantic surfclam, *Spisula solidissima similis*, which extends from Massachusetts through the Gulf of Mexico (Abbott 1974), does not occur in sufficient numbers to sustain a natural fishery. Preliminary studies based on growth rates observed in natural populations seem to indicate that the aquacultural potential for this subspecies is good (Walker and Heffernan 1994). Technical difficulties encountered while attempting to rear the southern Atlantic surfclam through larval and juvenile phases has in the past proved prohibitive to their development as an aquacultural candidate (Walker and O'Beirn 1996). Protocols have since been established whereby the successful culture of *S. s. similis* has been achieved through the larval phase and into the nursery phase of culture (Walker et al. 1995, Hurley 1996).

Logically, it would be seem that the next step would be to establish protocols for rearing the juveniles to a size (≈15 mm) large enough to relocate to field growout environments. As an initial step in this process, the growth of the southern Atlantic surfclam was evaluated under protocols established previously for both the Atlantic surfclam and the northern quahog, *Mercenaria mercenaria* (Goldberg 1980, Castagna and Kraeuter 1981). These

protocols would require the maintenance of juveniles of either subspecies in a temperature-controlled room (ca. 20°C) throughout the summer months (June to September; Walker and Hurley 1995). Ambient summer water temperatures in Georgia can exceed 30°C for extended periods, which have in the past proved lethal to *S. s. solidissima* (Walker and Heffernan 1994). This reports describes a study whereby the growth of the southern Atlantic surfclam, *S. s. similis*, was compared with that of the Atlantic surfclam, *S. s. solidissima*, under identical culture conditions.

METHODS

On March 11, 1995, Atlantic surfclam, *S. s. solidissima*, individuals were brought into the Shellfish Aquaculture Laboratory (SAL) of the University of Georgia Marine Extension Service on Skidaway Island, GA, from the winter holding cages at House Creek in Wassaw Sound, GA. These animals had all been spawned in the spring of the previous year (1994); thus, they were approximately 1 y old. On return to the laboratory, clams were divided into three separate groups ($n = 65, 65, 66$) to be used as broodstock.

On March 19, 1995, two groups ($n = 65, 66$) of the Atlantic surfclam broodstock spawned spontaneously. Larvae from these spawns were reared according to standard hatchery techniques (Castagna and Kraeuter 1981). Initial embryo stocking densities in the rearing tanks were 30 larvae/mL, which were reduced to 7.8 and 6.4 larvae/mL on attainment of the veliger stage of development. Throughout the larval stages, they were fed a ration of 50,000 cells of Tahitian strain *Isochrysis* sp. per milliliter per day, which was increased to 75,000 cells/mL per day when the larvae reached the veliger stage. On April 14, 1995, the first animals set. Most animals were set by April 21. The mean size at set was approximately 300 µm.

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On March 11, 1995, southern Atlantic surfclams, *S. s. similis*, were brought into the laboratory and were maintained under conditions similar to those of their Atlantic counterparts. They were approximately the same age as the Atlantic surfclams, having been acquired from the wild (St. Catherines Sound, GA) the previous year (1994) and aged as young-of-the-year (as per Walker and Heffernan 1994). The adults were conditioned until March 31, 1995, when they were induced to spawn with serotonin. The larvae were stocked in the larval tanks at 3.6 larvae/mL and were fed according to the regimen outlined above. Throughout their larval and most of their postset existence, both sets of subspecies were fed cultures of Tahitian strain *Isochrysis* sp. and *Chaetoceros muelleri*, individually and in combination. The southern Atlantic surfclam larvae were set by Day 23 (April 23, 1995), at a mean size of approximately 280 μ m.

Both sets of juvenile clams were maintained in raceways (3.6 \times 0.6 \times 0.3 m) until the commencement of the study. A daily feeding regimen was implemented for both sets of animals. However, because of the constraints imposed by the number of clams being cultured in the hatchery in 1995, food was often in short supply. It was necessary, on occasion, to sacrifice a daily feed for the Atlantic surfclam juveniles. This protocol did appear to retard the growth in the Atlantic surfclams, thus making the sizes of the animals from both species, at the start of the study, comparable.

On June 21, 1995, two tanks (400 L each) were set up in a thermally regulated (ca. 20°C) room at the SAL. Within each tank, 10 upwellers (ca. 40 cm in diameter) were assembled, with 1-mm-pore-size mesh screen bottoms. In each upweller, 40 g wet weight of juvenile surfclams was placed. In each tank, five upwellers contained individuals of *S. s. similis* and five contained *S. s. solidissima*. Each group of animals was assigned randomly to the upwellers. At the commencement of the study, the mean sizes of the Atlantic and southern Atlantic surfclams were 6.40 ± 0.11 (SE) and 6.43 ± 0.16 (SE) mm, respectively.

Each tank was drained on a daily basis, and each upweller and tank were cleaned and rinsed. The tanks were refilled with conditioned (at 20°C) mass algal cultures that had been pumped into the room the previous day. The food consisted of batch cultures of *C. muelleri*, Tahitian strain *Isochrysis* sp., and *Skeletonema* sp. Later in the study, food was supplemented with lyophilized *Isochrysis* and *Chaetoceros*. Every 2 wk, the contents of each upweller were weighed (gram wet weight) and the sizes of 30 animals were measured for maximum posterior-anterior distance (shell length). No sampling was carried out in Week 12. A repeated measures analysis was carried out, comparing the Atlantic surfclam wet weights and shell lengths with those of their southern Atlantic congeners, using analysis of variance and Tukey's Studentized Range Test ($\alpha = 0.05$). The study was terminated on October 10, 1995, 14 wk after its initiation.

RESULTS

Within each subspecies, no significant differences in shell lengths or wet weights were apparent between the two tanks. Consequently, the results of the two tanks were combined for each species. From the onset of this experiment, it was apparent that the Atlantic surfclam juveniles grew more rapidly than their southern counterparts (Figs. 1 and 2). By the first sampling period (Week 2), the proportional increase in wet weight of the Atlantic surfclam was more than double that of the southern surfclams (257 vs.

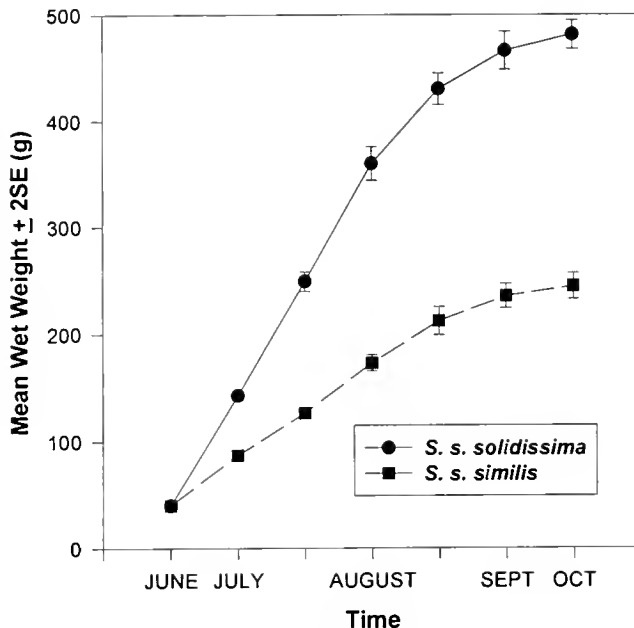


Figure 1. Mean \pm 2 SE, wet weights (g) of Atlantic surfclams, *S. s. solidissima*, and southern Atlantic surfclams, *S. s. similis*, obtained throughout the 14-wk grown trial.

118%). These differences in proportional increases between the two groups of animals decreased through subsequent samplings. Overall, however, the increases in the Atlantic surfclams (1.103%) were substantially greater than the corresponding increases in wet weight in the southern Atlantic surfclams (513%). The increase in wet weight was linear for both sets of animals throughout the first 6 wk of the study, after which there was a lowering in the rate of increase (Fig. 1).

No shell length differences existed ($p = 0.8707$) between *S. s. solidissima* and *S. s. similis* on initiation of the study. However, in the subsequent sampling periods, the repeated measures analysis revealed highly significant differences ($p < 0.0001$) between the two groups. The Atlantic surfclams were significantly larger throughout the study than the southern surfclams. Throughout the study, clam numbers in each upweller did not fluctuate drastically,

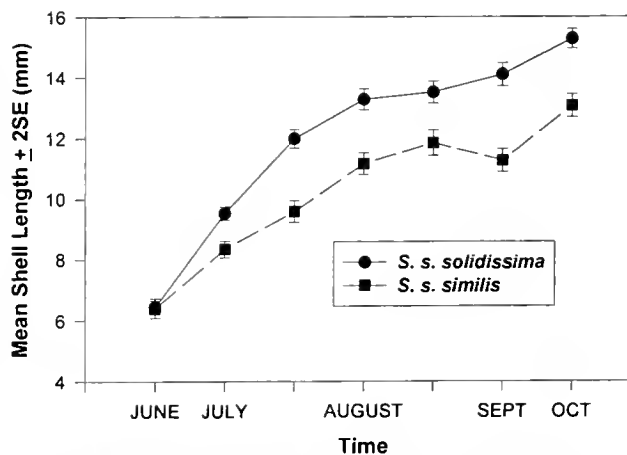


Figure 2. Mean \pm 2 SE, shell lengths (mm) of Atlantic surfclams, *S. s. solidissima*, and southern Atlantic surfclams, *S. s. similis*, obtained throughout the 14-wk growth trial.

and values in and around 1,000 clams per upweller were maintained. Consequently, we concluded that there was little or no mortality observed in this study.

DISCUSSION

The Atlantic surfclam, *S. s. solidissima*, performed markedly better than its congener, the southern Atlantic surfclam, *S. s. similis*, in our study. Over the 14 wk of our study, the mean increase in the size of the Atlantic surfclam was 8.9 mm, whereas that of the southern surfclam was 6.6 mm. The percent increase in biomass was 1,103% for *S. s. solidissima*, compared with a 513% increase for *S. s. similis*.

It is appreciated by the authors that the handling of the broodstock, larvae, and postset juveniles was different for the two species. However, on the basis of the literature pertaining to the culture of marine bivalves (Castagna and Kraeuter 1981), the southern animals were treated markedly better in terms of larval densities and feeding regimen than their more northerly counterparts, thus seemingly conferring an advantage on them. Inadvertent selection for hardier and more vigorous animals might have occurred with the Atlantic surfclams, given their low food rationing, compared with the more regular feeding regimen used for the southern surfclams before the study. Yet on the basis of the observations throughout the raceway stage of their culture, mortality did not appear to be high in the Atlantic surfclam cohort, suggesting that selection was not occurring. Having acknowledged this, the results are still surprising. The considerable growth exhibited by the Atlantic surfclam over that of the southern Atlantic surfclam was remarkable. The growth increases of *S. s. solidissima* (approx. 9 mm in 14 wk) achieved in this study were modest compared with the 12 mm in 3 wk obtained by Goldberg (1980), who cultured juvenile Atlantic surfclams with unfiltered seawater in a flow-through system. However, given the limitations imposed by low food quantities in this study (as attested by the rapid clearing of the water subsequent to feeding in both tanks), the differences observed between the two studies (Goldberg 1980, this study) were not surprising. The reduction in the growth rate imposed by apparent low food rations is clearly visible in the respective figures relating to both clam biomass and size. After 6 wk, there was a noticeable reduction in the increases in clam biomass values (Fig. 1) and growth rates (Fig. 2). The finding that this reduction was similarly observed in both *S. s. similis* and *S. s. solidissima* suggests that this particular phenomenon was an artifact of suboptimal environmental condi-

tions (i.e., low food). However, the differences in the responses of the congeners to similar environmental conditions throughout the study do suggest that some basic physiological differences exist between the two, under the conditions of this study. Given the differences documented elsewhere between adults of these species, the results of this study might not be as surprising as they seem. The Atlantic surfclam does differ from the southern surfclam in a number of life history characteristics, e.g., maximum size and age, as well as timing of gametogenesis (Abbott 1974, Ropes and Ward 1977, Sephton and Bryan 1990; Kanti et al. 1993, Spruck et al. 1994, Walker and Heffernan 1994).

A costly, yet critical portion of operating a bivalve mollusk hatchery/nursery is the rearing of the animals from the larval stages through juvenile stages, until such a time as they are large enough to plant in the field. A consistent source of quality water, as well as food, is required throughout this phase. This can financially stress even the most efficient of operations. The problem is exacerbated even further if the animals require water temperature other than that of ambient conditions. Given that the optimal temperature for rearing larval *Spisula* sp. has been determined to be in the region of 20°C (Walker and Hurley 1995), that temperature was chosen for the nursery stage in the trials described here. If a facility is constrained by such temperature requirements, the ideal situation would be to grow these animals to planting sizes as soon as possible and, therefore, minimize nursery costs. The sizes achieved for both sets of animals at the end of the study were sufficient to plant in the field such that survival and growth would be maximized (Goldberg and Walker 1990). One apparent advantage the southern Atlantic surfclam has over the Atlantic surfclam is their ability to withstand higher water temperatures, such as those experienced in summer in coastal Georgia. This advantage would enable the culturist to plant the southern animals in the field earlier (September), when the water temperatures are still high enough (approx. 28°C) to induce mortality in the Atlantic surfclam juveniles. Yet, the feasibility of such a protocol would still have to be evaluated, given the relatively small sizes of the southern animals during September (Fig. 2).

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JUVENILE AND YEARLING GROWTH OF ATLANTIC SURFCLAMS *SPISULA SOLIDISSIMA* (DILLWYN, 1817) IN MAINE

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ABSTRACT With the recent emergence of a shellfish aquaculture industry in Maine, the development of alternative species would provide mariculturists some flexibility and stability by diversifying their product line and opening up coastal environments unsuitable to the oysters and mussels currently being cultivated. The Atlantic surfclam, *Spisula solidissima*, occurs naturally in Maine, and although it has not been commercially exploited, this macruid clam may provide growers with a profitable new product line. What is not known is how well this species will grow in a culture setting throughout Maine's diverse marine environment. The goal of this study was to assess the growth and survival of two age/size classes of Atlantic surfclams under a variety of growing conditions. Juvenile (3-mm) and yearling (23-mm) surfclams were reared for one growing season in floating screened trays and intertidal sediments, respectively, at six study sites along the coast of Maine. After 4 mo of growth, mean size differences of juveniles among the six growing sites were significant. Juveniles reared at the upper Damariscotta River site grew the fastest (8.9 mm shell length [SL]) among the six sites. In comparison, those grown in Mud Hole Cove had the slowest growth (5.5 mm SL). Yearling surfclams at both planting densities grew the fastest in the Mud Hole Cove plot (40 mm SL) compared with the slowest growing sibling cohorts in the Deer Isle plot (27 mm SL). Similar trends among plots were observed with respect to both wet and dry weight gain. Surfclams reared in low-density treatments tended to grow faster than the high-density cohorts, although the means were not significantly different at any of the study plots. The optimal nursery sites for juvenile growth were different from the most productive areas for yearling growth, suggesting that growers may want to choose separate areas for different culture phases. This study is the first to document rates of growth and survival of Atlantic surfclams reared under varying growing conditions in northern New England waters.

KEY WORDS: Atlantic surfclam, growth, site selection, aquaculture

INTRODUCTION

The Atlantic surfclam (*Spisula solidissima*) is a subtidal macruid species ranging in distribution along the eastern seaboard from Labrador, Canada, to South Carolina (Abbott 1974). Also referred to as the bar or hen clam, this species can grow to over 157 mm in shell length (SL) and typically inhabits sandy environments from just beyond the surf zone to deeper, offshore waters. A commercial fishery exists off the Middle-Atlantic Bight for 100–125 mm SL surfclams. Commercial landings of Atlantic surfclams in 1993 in the United States were 33,600 metric tons, valued at nearly \$34 million (NOAA/NMFS 1994, Murawski et al. 1990). In recent years, interest has grown to evaluate the aquaculture potential of this species.

Goldberg and Walker (1990) assessed the growth and survival of cage-cultured yearling surfclams in the waters of Georgia to determine if this species would tolerate the southern waters beyond its natural southern range. They determined that growth rates of surfclams reared in coastal waters were greater than those grown in nearby intertidal rivers. Research by Walker and Helfernan (1990a, 1990b) assessed the effects of cage mesh size and planting height on growth and survival of surfclams in the coastal waters of Georgia. Mesh size had no effect on survival, and clams grew faster and had higher survival rates when planted lower in the intertidal zone.

Several studies have been undertaken to determine the mariculture potential of this species in New England waters. Goldberg

(1980, 1989) assessed the potential of rearing surfclams in a race-way system in Milford, CT. In this study, juveniles grew from 18 to 55 mm in one growing season, suggesting that one could rear them to market size within 1 y, although growth in the natural environment could be considerably different. No studies to date have assessed the mariculture potential for this species in northern New England waters.

With the emergence of a shellfish aquaculture industry in Maine, the development of alternate species such as the Atlantic surfclam would provide mariculturists some flexibility and stability by diversifying their product line and possibly opening up new growing areas that are presently unsuitable for the species currently in culture. One Maine aquafarmer has been successfully rearing surfclams to 45 mm SL in 2 y. This size of product could compete with the cherrystone hard clam market.

The purpose of this study was to assess the growth of two age/size classes of Atlantic surfclams in various growing environments along the coast of Maine. Clams were reared for one growing season in floating screened trays (juveniles) or intertidally in sediment-filled containers (yearlings) at two planting densities at six study sites spanning the Maine coastline.

MATERIALS AND METHODS

Juvenile Growth Study

Juvenile surfclams measuring approximately 3 mm in SL were acquired from a commercial shellfish hatchery (Mook Sea Farm,

Inc., Damariscotta, ME) in June 1992. The genetic heritage of the parental broodstock is unknown. Spawning, larval rearing, and early nursery growth occurred from April through June 1992. Juveniles were deployed in six intertidal plots along the coast of Maine (Fig. 1). They were initially reared in floating screened trays similar to those used by commercial growers. Tray assemblies consisted of 31 × 76 cm mesh envelopes supported by 36 × 81 cm rectangular frames made of 12.7-mm-diameter polyvinyl chloride pipe. Mesh envelopes were made of either fiberglass window screen (1-mm mesh size) or polyethylene netting (4.2-mm mesh size), depending on the size of the surfclams. The mesh envelope and frame assemblies were contained in 46 × 81 × 9 cm extruded polyethylene cages (ADPI OBC-3 cage with 12.7-mm mesh size). Each tray was fitted with foam flotation providing 3.6 kg of buoyancy. Trays were tied end-to-end and secured by a single-point mooring. Approximately 20 periwinkles (*Littorina littorae*) were stocked with the surfclams to help control biofouling. Trays were also scrubbed of fouling organisms as needed. Three replicate trays were placed at each study site, and surfclams were sampled monthly to estimate growth and mortality.

SL were initially measured by video image analysis. Subsequent monthly growth measurements used digital calipers (± 0.1 mm) once the clams were large enough to be handled safely. Surfclams were deployed from June 12–22, 1992, at six intertidal sites from the Piscataqua River, York County, on the western Maine coast to Mud Hole Cove, Great Wass Island, Washington County, to the east (Fig. 1). Replicate trays were initially stocked with 430 individuals. Floating tray sites were located as close as possible to the intertidal bottom sites. Monthly sampling occurred from July to October 1992. Individuals were randomly sampled (without replacement) for subsequent measurement of shell length ($n = 24/\text{tray}^{-1}$).

Bottom Growout of Yearlings

The experimental design for the yearling surfclam growout study used 48 sediment-filled containers per study plot: one-half (24) were stocked with surfclams at a density of 12/unit (high density), and the remaining 24 were stocked at a density of 6/unit (low density). These high- and low-density treatments equate to 658 and 329/m², respectively. The design was replicated at each of the six study sites, yielding a total of 288 experimental units containing 2,592 surfclams. Fourteen-month-old surfclams were acquired from Mook Sea Farm, Damariscotta, ME. They had been reared in floating screened trays in the Damariscotta River the

prior summer, were wet stored over the winter, and were then made available for this project. Before deployment, all clams were measured to determine initial SL (23 mm) and live weight (LW) (2.1 g). Surfclams were randomly allocated at the above-prescribed densities to each of 48 numbered experimental units for each of the six plots. Growing containers consisted of 15.2-cm-diameter by 15.2-cm-deep plastic flower pots filled to the brim with sediment from the mud flat adjacent to the experimental array within each study site. Although sediment type varied from site to site, it was homogeneous within the arrays at each site. Each pot was covered with polyethylene predator netting (4.2 mm mesh size), secured to the containers by heavy rubber bands. The containers were then buried in the mud flat with the tops flush with the surrounding sediment. Twenty-four low-density and 24 high-density treatments were randomly deployed in an 8 by 6 array adjacent to the water's edge at mean low water (MLW). Experimental arrays at each of the six study sites were thus submerged for an equivalent proportion of the tidal cycle. The location of each numbered container was noted for future retrieval. Monthly sampling from July through October 1992 consisted of randomly removing without replacement six experimental units from each of the two density treatments for subsequent measurement. Predator netting on the unsampled experimental units were checked for excessive fouling and cleaned as necessary. Mensuration entailed determination of shell height, LW, dry weight, and dry tissue weight. Counts of dead and/or missing individuals were noted. Dry tissue weights were determined by shucking the meats of each individual into a pretared weighing boat and drying to constant weight at 70°C.

Statistical analysis was done with the SYSTAT statistical package (Wilkinson et al. 1992). Analysis of variance (ANOVA) was used to test for significant differences in SL, LW, dry weight, and dry tissue weight among cohorts or plots at each study site. The Tukey-Kramer HSD *post hoc* test for mean separation was used to further discriminate significant ($p < 0.05$) differences among cohort means between plots. The Student *t*-test was used to detect significant size differences due to density effects.

Descriptions of the Study Sites

Piscataqua River

This intertidal site is located on the northern shore of the Piscataqua River in the town of Eliot, York County (latitude, 43°05.7'N; longitude, 70°46.4'W) (Fig. 1). The study site is adjacent to the mouth of Spinney Creek, an artificially impounded bay that has historically been used for shellfish aquaculture. Current velocities in the Piscataqua River often exceed 180 cm/sec on the ebb tide. This portion of the river has a 2.6-m mean tidal range, and the steep gradient of the intertidal zone results in a narrow band of sediments varying from firm sand to coarse gravel. The experimental array was sited within the sandy portion of the beach-front.

Maquoit Bay

This site is located along the western shore of Maquoit Bay in the town of Brunswick, Sagadahoc County (latitude, 43°51.1'N; longitude, 70°01.8'W) (Fig. 1). Extensive mud flats at low water are covered by patches of eel grass (*Zostera marina*). The mean tidal range is 2.9 m. Intertidal sediments vary from soft mud to coarse sand proceeding up the intertidal zone. The experimental plot was located in soft mud. Maquoit Bay has historically sup-

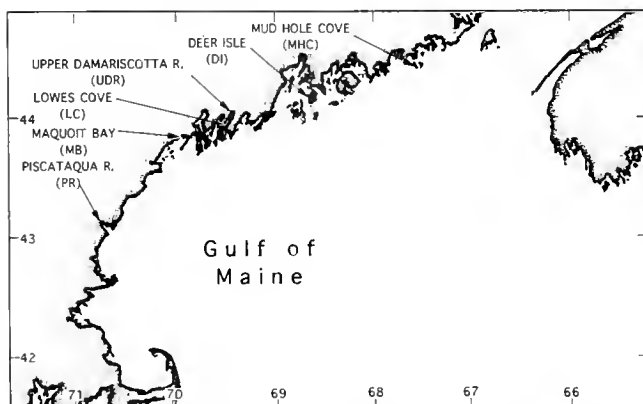


Figure 1. Location of the six study sites in the Gulf of Maine.

ported large populations of soft-shell clams (*M. arenaria*), although a massive (30–40%) mortality of clams was observed in 1988 due to anoxic conditions after an unusually large dinoflagellate bloom (Heinig and Campbell, 1992).

Lowes Cove

Lowes Cove is located along the eastern shore of the Damariscotta River in the town of South Bristol, Lincoln County (latitude, 43°56.1'N; longitude, 69°34.6'W) (Fig. 1). The cove supports a soft-shell clam (*M. arenaria*) population in the very soft mud sediment found throughout the cove. The study site was located at the mouth of the cove and, because of the southwest exposure, was subject to considerable wave action from the prevailing summer southwesterly winds.

Upper Damariscotta River

This study site is located along the western shore of the Damariscotta River near the head of navigable waters in the town of Newcastle, Lincoln County (latitude, 44°01.8'N; longitude, 69°32.4'W) (Fig. 1). Extensive mud flats extend from the upper shore for several hundred meters. The upper portion of the Damariscotta River is highly regarded among aquaculturists because of its high productivity and warm waters during the summer months. A shellfish aquaculture nursery lease is located nearby. One nearby grower has been successfully rearing Atlantic surfclams in floating nursery trays and bottom cages for several years.

Deer Isle

Located at the southern entrance to Mud Cove on Stinson's Neck, Deer Isle, Hancock County (latitude, 44°12.6'N; longitude, 69°34.2'W), this site lies adjacent to a bottom culture mussel farm (Fig. 1). Sediments consist of a mixture of fine sand and soft mud interspersed with large boulders and outcrops of bedrock. The site has a mean tidal range of 3.3 m and has a southeasterly exposure.

Mud Hole Cove

This intertidal site is located in the upper reaches of Mud Hole Cove, Great Wass Island, Washington County (latitude, 44°27.5'N; longitude, 67°35.4'W) (Fig. 1). This long and narrow protected cove has intertidal sediments consisting of soft mud (mean tidal range of 3.5 m). Mud Hole Cove is also the nursery area for a nearby shellfish hatchery.

Environmental Monitoring

Monthly surface water temperature, salinity, and chlorophyll *a* measurements were taken at each of the study sites on the dates that the surfclams were sampled for growth and survival. Water samples were taken near the time of MLW. In addition, weekly chlorophyll *a* measurements were made at the two Damariscotta River sites at varying stages of the tidal cycle. Temperature ($\pm 0.5^\circ\text{C}$) was measured with a mercury thermometer. Either an optical refractometer or a hydrometer was used to determine the salinity ($\pm 0.5\text{‰}$). Replicate water samples ($n = 2$) were gathered from 0.25 m below the surface. Levels of chlorophyll *a* were determined with a Turner Model 110 fluorometer, following the methods of Strickland and Parsons (1972).

RESULTS

Juvenile Growth

Juvenile growth in the floating screened trays varied considerably from site to site. Among the six sites, surfclams reared at the upper Damariscotta River site grew the largest by the end of the growing season in October (SL \pm SD) of 8.94 [1.98] mm (Fig. 2). In contrast, surfclams in Mud Hole Cove grew the slowest in the same time period (5.48 [1.17] mm SL). Surfclams at the remaining three areas grew to a mean size of 5.98 (0.13), 7.41 (0.32), and 6.64 (0.17) mm SL for the Piscataqua River, Maquoit Bay, and Lowes Cove sites, respectively. The experiment in Deer Isle was terminated when the three experimental trays were inadvertently lost from their mooring sometime between mid-July and mid-August.

ANOVA indicated highly significant ($p < 0.001$) differences in SL for clams in October. Tukey HSD multiple comparison tests showed that clams were significantly larger ($p < 0.05$) at the Upper Damariscotta River site than at all other sites. Clam size was not significantly different between Maquoit Bay and Lowes Cove or between the Piscataqua River and Mud Hole Cove sites (Fig. 2). Clam length was also not significantly different between the Lowes Cove and the Piscataqua River stations. Similar Tukey HSD multiple comparisons were made for the earlier sampling dates, and as would be expected, differences in size among sites became more pronounced as the growing season progressed.

Figure 3 illustrates the instantaneous growth rates for juvenile surfclams at five of the sites throughout the study period. Growth rates were greatest ($k > 1.25$) at all study sites during July and steadily declined as the summer progressed ($k < 0.5$). Mortality of juveniles was nil over the course of the study.

Yearling Growth

Considerable variation in mean growth rate of yearling surfclams was observed among plots. Growth occurred throughout the study period but was generally greater during July and August. The greatest growth in SL by October was observed at the Mud Hole Cove plot in both the high- and the low-density treatments. Mean sizes (\pm SD) for these groups were 40.2 (1.40) and 38.8 (3.49) mm, respectively. Surfclams reared at the Deer Isle plot had slowest

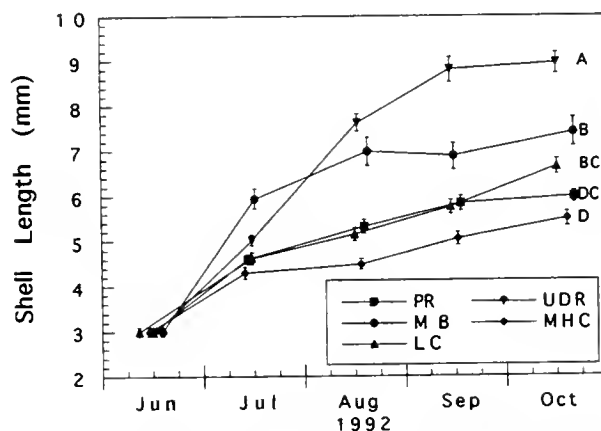


Figure 2. Changes in SL of juvenile surfclams at five study sites in Maine. Error bars indicate ± 1 standard error. October means with differing letters are significantly different ($p < 0.05$) from one another. See Figure 1 for explanation of abbreviations.

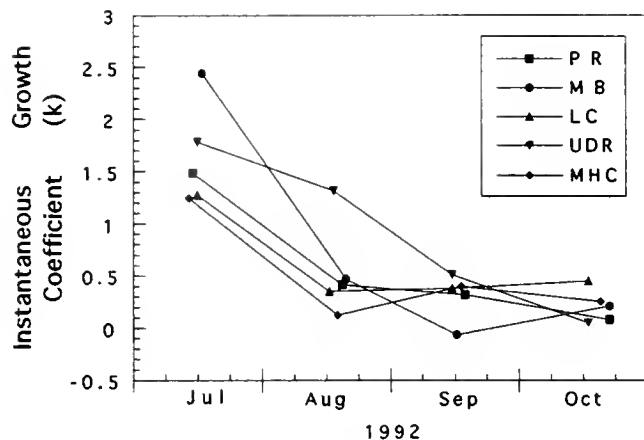


Figure 3. Plot of instantaneous growth rates for SL of juvenile surfclams reared at five study sites. Note that the plotted values are determined at the end of each sampling interval, but reflect the average growth rate for the preceding month.

growth among the six study plots in both the high- and the low-density treatments (27.1 [0.55] mm and 27.6 [1.34] mm SL, respectively). October mean sizes for the remaining high-density treatments were 39.4 (1.44), 37.3 (0.64), 33.7 (0.99), and 28.4 (1.39) mm SL at the Lowes Cove, Upper Damariscotta River, Maquoit Bay, and Piscataqua River plots, respectively. Corresponding low-density means were 37.5 (2.30), 38.6 (2.18), 32.8 (2.30), and 27.0 (2.16) mm SL, respectively. Changes in mean SL over the course of the experiment for the high- and low-density treatments for each of the plots are illustrated in Figure 4. ANOVA indicated that differences in mean SL among the six plots were highly significant ($p < 0.001$), although density and density \times plot interactions were not ($p > 0.05$). Tukey HSD multiple comparison tests on size of surfclams cultured at both densities give similar results (Fig. 4). Mean clam sizes at the Upper Damariscotta River, Lowes Cove, and Mud Hole Cove plots were not significantly different from one another, but were significantly larger than clams from Maquoit Bay. Clams from Maquoit Bay were significantly larger than those from Deer Isle and Piscataqua River plots, which were not significantly different from each other (see Figure 4).

Similar comparisons were made for the LW data. By October, surfclams reared at high density at the Lowes Cove and Mud Hole

Cove plots grew to 11.6 (1.29) and 11.5 (0.86) g mean LW, respectively. (The difference in weight between these two groups is not significant [$p > 0.05$].) The corresponding low-density treatments were 10.1 (1.39) and 11.1 (0.99) g LW. In comparison, surfclams from the Deer Isle plot only grew to 3.8 (1.24) and 4.0 (1.29) g LW for the high- and low-density treatments, respectively. October mean LW were 9.9 (0.49)/10.8 (1.98), 6.6 (0.56)/6.2 (1.17), and 4.1 (0.57)/3.7 (0.92) in the high-/low-density treatments for the Upper Damariscotta River, Maquoit Bay, and Piscataqua River plots, respectively. Figure 5 illustrates the changes in LW throughout the study period for the high- and low-density cohorts, respectively.

ANOVA indicated that differences in mean LW among the various plots were highly significant ($p < 0.001$). Similar results were observed for clams reared at high and low densities. Tukey HSD multiple comparison tests indicated that for October, there was no significant difference in the mean weight of surfclams between the Deer Isle and Piscataqua River plots or between clams from the Upper Damariscotta River, Lowes Cove, and Mud Hole Cove plots. The mean LW of clams from Maquoit Bay were statistically different ($p < 0.05$) from those of clams from all other plots (see Figure 5).

Mean dry animal and tissue (meat) weights were determined for cohorts for each plot (see Figure 6). ANOVA for each of these parameters were highly significant (both, $p < 0.001$) with respect to growing plot. Subsequent multiple comparisons based on mean dry tissue weight indicated that the Mud Hole Cove cohort was significantly greater ($p < 0.05$) than the other groups (0.640 [0.060] and 0.611 [0.079] g for high and low groups, respectively). The Maquoit Bay and Deer Isle groups had the lowest mean dry tissue weights (0.119 [0.017]/0.111 [0.041] and 0.126 [0.012]/0.133 [0.023] in the high/low treatments, respectively), but the means were indistinguishable from each other.

Combined cumulative mortality for all six plots by October was 0.81%. Cumulative mortality at individual study plots varied from 2.8% (Upper Damariscotta River) to <1.0% (Piscataqua River, Maquoit Bay, Lowes Cove, and Mud Hole Cove).

Environmental Data

Peak summer temperatures varied considerably from site to site. The Upper Damariscotta River site had the highest maximum surface water temperature ($>20^{\circ}\text{C}$, June to August), whereas Mud

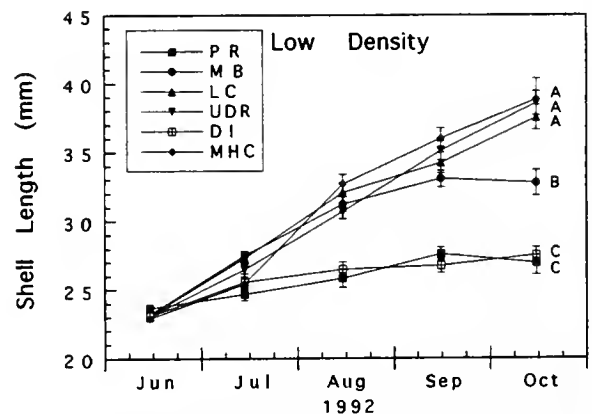
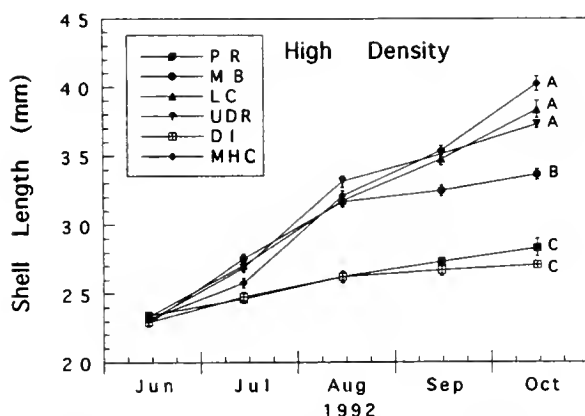


Figure 4. Changes in SL of yearling surfclams reared at high and low densities at six sites in Maine. Error bars indicate ± 1 standard error. October means with differing letters are significantly different ($p < 0.05$) from one another.

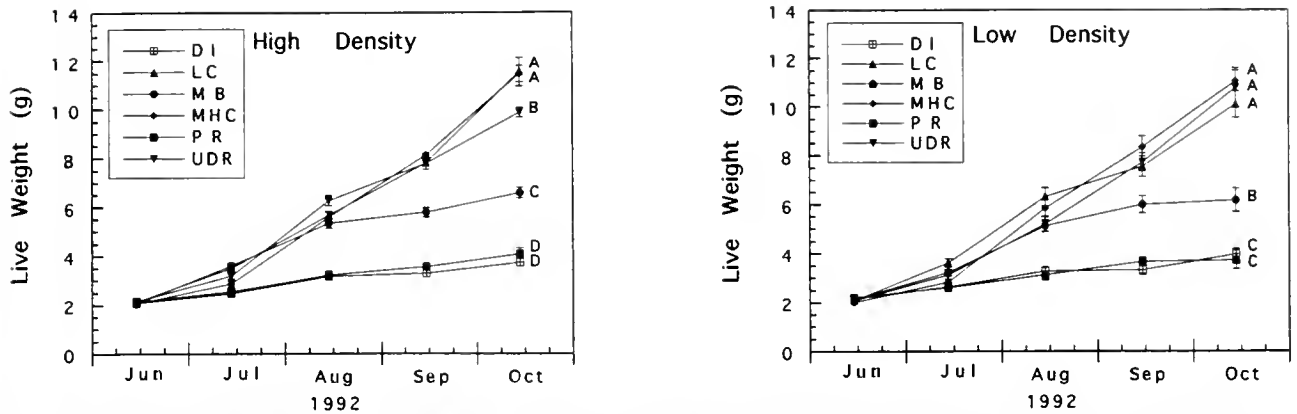


Figure 5. Changes in LW of yearling surfclams reared at high and low densities at six sites in Maine. Error bars indicate ± 1 standard error. October means with differing letters are significantly different ($p < 0.05$) from one another.

Hole Cove had the lowest maximum temperature of 13°C in June and July (see Figure 7, dashed lines). Temperatures dropped precipitously at all sites after mid-September. Salinities from all of the study sites ranged from 30 to 34‰ (Fig. 7, solid lines) and never varied by more than 2‰ at any site. As would be expected, the two estuarine sites (Piscataqua and Upper Damariscotta Rivers) had slightly lower salinities than the more oceanic locations.

Concentrations of chlorophyll *a* may provide an approximation of the food available in the water column for shellfish consumption. The Upper Damariscotta River site had the highest chlorophyll *a* values ($6.8 \mu\text{g/L}$), whereas the Piscataqua River and Deer Isle sites had the lowest levels (Fig. 8). An early summer peak followed by an August crash was seen in the Upper Damariscotta River and is typical for that area (C.R. Newell, Pers. comm.). A similar profile was seen at the Deer Isle and Piscataqua River sites.

DISCUSSION

Significant variation in growth rates of Atlantic surfclams was observed among the six study plots. The relatively rapid growth of yearling cohorts from the Mud Hole Cove and Lowes Cove plots suggests that these areas may be superior locations for yearling growth of surfclams. Replication of the study plots within each site would be required to extrapolate these findings for the area in question. Interestingly, the least productive nursery site for juve-

nile growth (Mud Hole Cove) was one of the most productive sites for yearling growth, suggesting that growers may want to choose separate areas for different culture phases. The food quantity and quality, as well as temperature regimens of waters over the benthic intertidal zone, may vary considerably from subtidal surface waters several meters away.

Planting density had no significant effect on any of the growth parameters measured at any of the study plots. Apparently, within the $329\text{--}658 \text{ individuals/m}^2$ range, planting density has little effect on growth rate. In comparison, Goldberg (1989) did observe density-dependent effects for surfclams reared in bottom cages. Clams reared in Connecticut from June through November increased in mean size from 15.7 mm to 47.3, 40.8, and 32.0 mm in the 500, 1,000, and 2,000 clams/ m^2 density treatments, respectively, thus suggesting that growth rate was inversely proportional to planting density under those growing conditions. It is possible that the lack of density-dependent effects in the Maine groups were due to the relatively low stocking densities compared with those in the Goldberg study. A comparison of the highest growth rates observed in the Goldberg (1989) low-density treatment (500 clams/ m^2) to surfclam growth in this study indicates that the greatest yearling growth in the Mud Hole Cove plots (23 mm initial size to 40.2 mm by October at $658 \text{ individuals/m}^2$) were still less than those seen in the Connecticut study (15.7–47.3 mm).

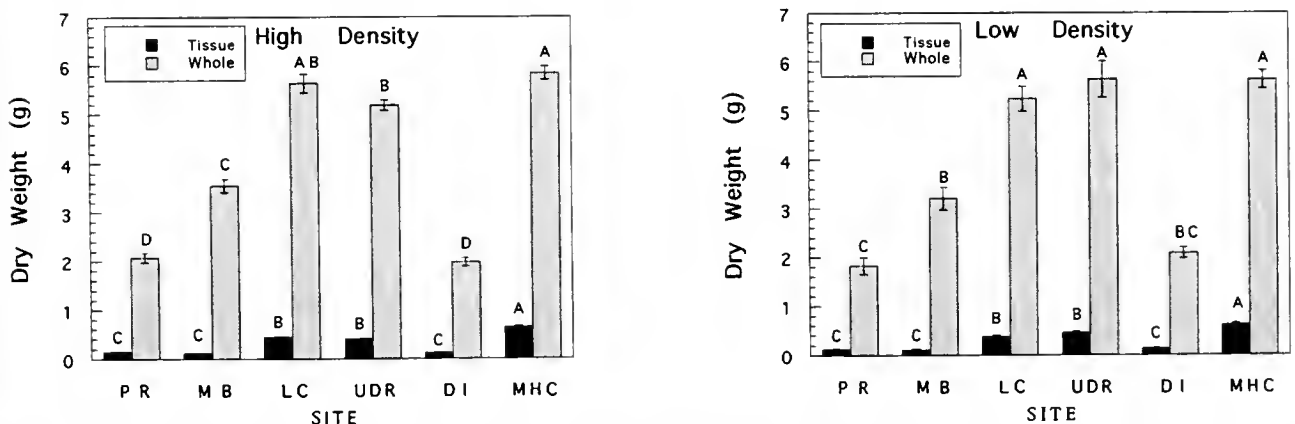


Figure 6. Dry whole-body and tissue weights for surfclams reared at high and low densities at six sites in Maine. Error bars indicate ± 1 standard error. Means with differing letters are significantly different ($p < 0.05$) from one another.

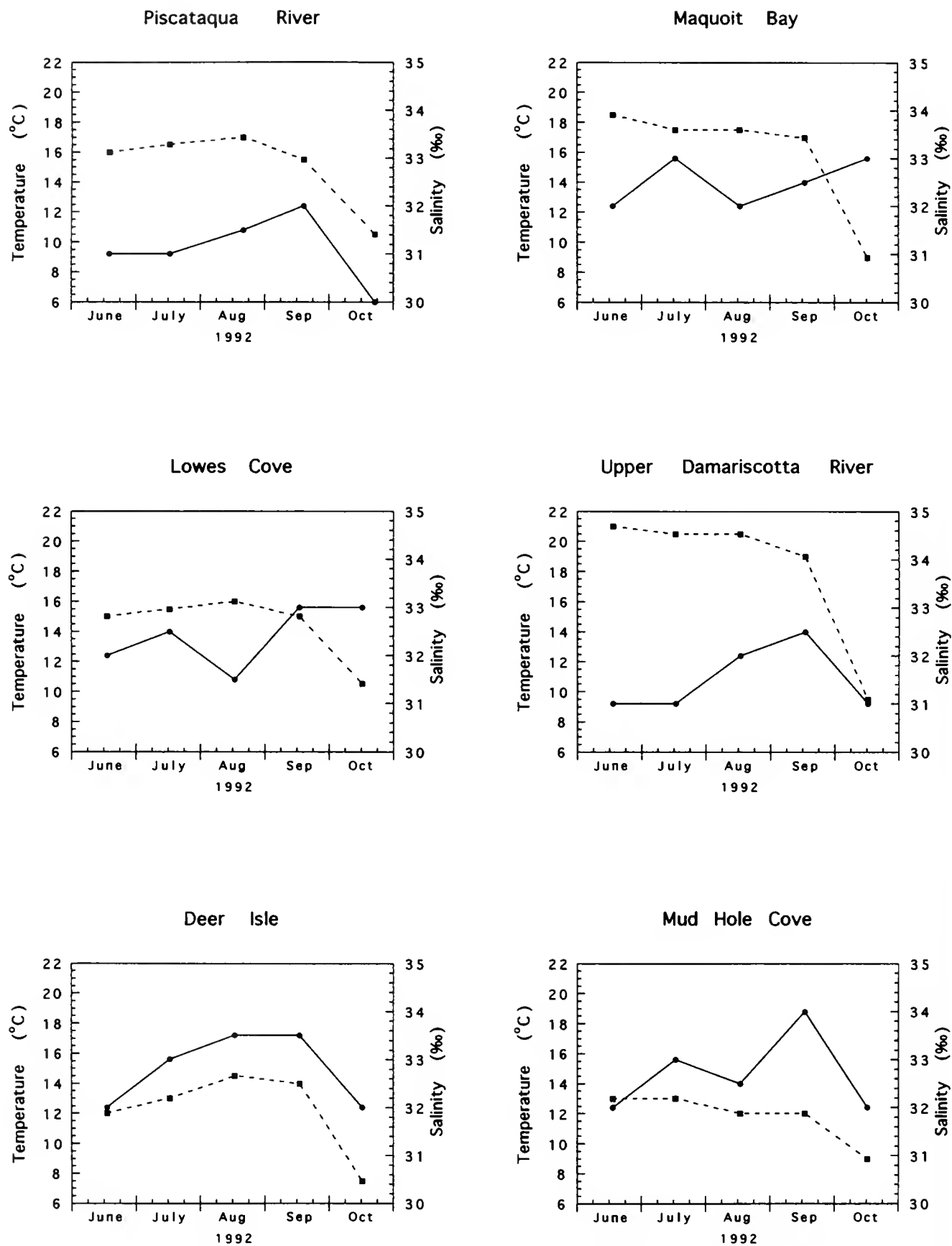
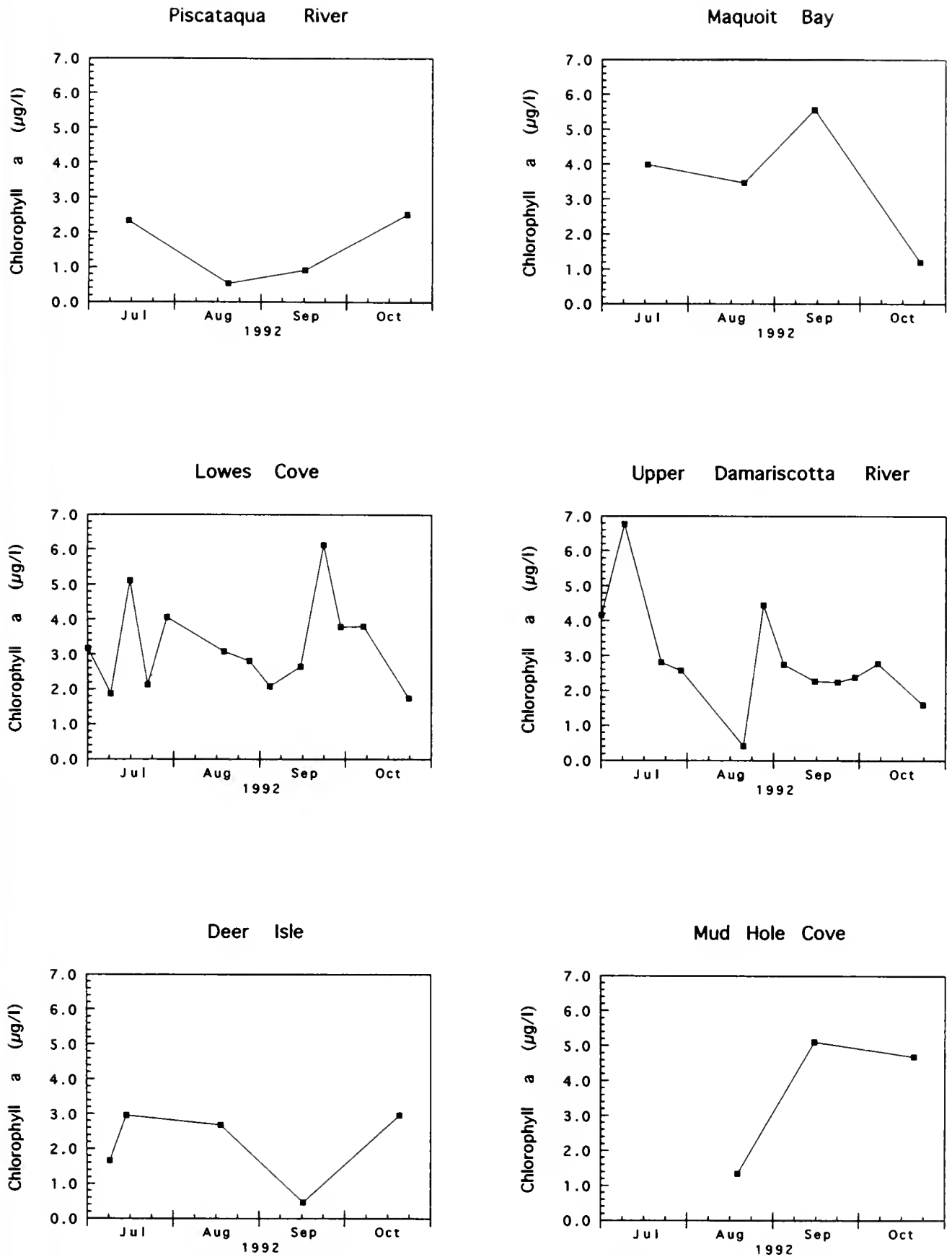


Figure 7. Temperature (°C) (dashed line) and salinity (‰) (solid line) profiles at the six field sites in Maine.

Figure 8. Chlorophyll *a* profiles at the six field sites in Maine.

Those sites with warmer water temperatures and higher levels of chlorophyll *a* tended to be associated with faster growing surfclams of both size/age classes (e.g., Upper Damariscotta River). In contrast, the poorer growth performance seen in the Piscataqua River and Deer Isle plots may reflect the lower chlorophyll *a* and water temperature profiles observed. All of the sites chosen for this study had stable and high summer salinities. The Atlantic surfclam is considered a stenohaline species and may not tolerate the lower springtime salinities of the riverine sites. Year-round environmental monitoring along with growth and survival trials for a proposed site is recommended.

The nature of the experimental containers (flower pots) for the yearling growth study clearly does not reflect the growout system that would be used in a production operation, but because of the intensive sampling nature of this project, the containers made the retrieval process a more manageable and less destructive exercise. Growth rates and survival could be very different if surfclams were to be directly seeded into a mud flat or reared in cages on the bottom. Furthermore, predator protection and harvesting ease must be considered when evaluating these methods. We only considered yearling growth in the low intertidal zone, but subtidal growout may be beneficial under conditions such as when there is interference with an existing intertidal shell fishery. Growth rates may also be enhanced in a subtidal culture setting, although predation problems may offset these gains. Goldberg (1989) showed that yearling surfclams grew faster at an 8-m depth versus shallower areas. This is an area needing further research.

Floating screened nursery trays are commonly used by shellfish mariculturists worldwide, but recent observations from experiments rearing another mactrid, Stimpson's surfclam (*Mactromeris polynyma*), in surface trays suggest that growth may be retarded compared with that of juveniles reared in sediment (C.V. Davis, unpubl. data). Surfclams spent considerable time (and presumably energy) foot probing the screen surface, presumably trying to burrow into the nonexistent sediment. Early planting of juvenile surfclams in sediment may be technically problematic, but on the basis of the *Mactromeris* data, growth rates may be enhanced.

This study underscores the importance of selecting an appropriate shellfish-rearing site, as indicated by the high variability of growth rates seen among the various growing areas. Undoubtedly, many variables beyond production-related ones will come into consideration when choosing a shellfish culture site. Factors such as access, protection, and compatibility with existing fisheries, etc., must be balanced with the need for a site with optimal growing and survivability conditions. The prudent mariculturist will evaluate several sites in a pilot study before beginning production.

ACKNOWLEDGMENTS

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HISTOCHEMICAL AND X-RAY STUDIES ON TISSUE CONCRETIONS AND SHELLS OF *MARGARITIFERA MARGARITIFERA* (LINNAEUS)

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ABSTRACT Concretions of two types, calcified (calcium and phosphorus containing) and lipofuscin containing, were found to occur in the tissues of the freshwater pearl mussel, *Margaritifera margaritifera* (L.), in Finland. Both concretions can also contain Fe^{3+} , and the calcified concretions can contain manganese and sometimes Fe^{2+} . The calcified concretions comprise (1) small spherules in the mantle and simple or combined spherules above the gill axes and the pericardial glands, in the gills, and among the heart muscle cells, (2) supporting rods in the gills, and (3) very small granules between the Leydig cells and body muscles. The mussel effectively concentrates calcium even from very soft water and stores it mainly in the mantle as extensive masses of small spherules. Large concretions (10–15 μm) in the pericardial gland cells of the pearl mussel may be an effective means for the concentration and excretion of lipofuscin and iron. Similar but smaller concretions can be found also elsewhere in the mussel, especially in the kidney. In the glochidial shells of the *Anodonta* species, calcium is mostly in the form of carbonate. Because the glochidial shells of *M. margaritifera* are thin and poorly calcified, some phosphorus and sulfur in the X-ray spectra may come from the larval mantle or the organic matter of the shell. Iron and manganese from mussel concretions do not markedly enter glochidial or adult shells. The precipitate on the adult *M. margaritifera* shell, however, contains large amounts of these elements.

KEY WORDS: Freshwater pearl mussel, *Margaritifera margaritifera*, *Anodonta*, calcium, iron, metal, concretion, lipofuscin, glochidium, shell

INTRODUCTION

The freshwater pearl mussel, *Margaritifera margaritifera* (L.), has previously been considered to be a "calciphobe" mussel. Differing from *Margaritifera auricularia* (Spengler), *M. margaritifera* has been believed to live in waters with little calcium. According to Björk (1962), *M. margaritifera*, however, also tolerates hard waters. Adult freshwater pearl mussel specimens have also been found to have a wide tolerance of water quality (Valovirta 1995a). This is important in the conservation programs of this species in Finland (Valovirta 1984, Valovirta 1995b). The ability to gather calcium ions in hard and soft waters as well would help the adult specimens of *M. margaritifera* to live over unsuitable periods of water quality (cf. Heming et al. 1988, Pynnönen 1990).

In the Ähtävä River, which has soft water, transplantation experiments with *M. margaritifera* were undertaken in 1987–1988 (Valovirta 1987). The mantles of the endemic mussels and the transplants were found then to be strikingly opaque and beige. This observation, and the fact that unionaceans commonly have calcium-containing granules in their tissues (e.g., Pynnönen et al. 1987) led to a histochemical study, in which the distribution of calcium-containing concretions was determined in this species.

High iron concentrations in river waters are detrimental to freshwater pearl mussels because they cause hardening of the sediment on river bottoms (Valovirta 1987). Iron and other components of different kinds of concretions in the tissues of the mussel were also studied histochemically.

Because the elementary composition of the concretions and the

glochidial shells of this species was not known, as a starting point for understanding the physiological roles of different kinds of concretions and the hard tissues, X-ray microanalyses were made from the concretions in the soft tissues, as well as from glochidial and adult shells. The results from the gill concretions and glochidia of *Anodonta anatina* (L.) and *Anodonta cygnea* L. were used for comparisons.

MATERIALS AND METHODS

About 20 mussels (*M. margaritifera*) were collected from the Ähtävä River, in western Finland (Kokkola Water and Environmental Board), in July 1988. The river water was very soft (total hardness < 1° dH), and its iron concentration was about 0.5 mg/L. The mean water pH is usually about 6.5, but during flood periods, it may occasionally drop below 5.0 (Valovirta 1987). The mussels, with shell lengths of 101–124 mm, were transferred in water to the Department of Biosciences, Division of Animal Physiology, University of Helsinki, and kept in charcoal-filtered, running, and aerated tap water (12°C) for a few days before sampling. The tap water was also relatively soft (18 mg of Ca/L).

A gravid female freshwater pearl mussel was collected from the river in September 1993, kept in an aquarium (at 7°C) by the Kokkola Water and Environment Board, and transported by air, wrapped in cold, moist paper, to Helsinki (October 1). The mussel was sampled immediately after it arrived.

The mussels were opened, and transverse slices were cut from the bodies with sharp, disposable knife blades. The slices were prefixed in a neutral glutaraldehyde-formaldehyde mixture (1:4, diluted in tap water according to Howard and Smith 1983), Lillie's

buffered formalin, or Bouin's fluid (see Table 1) for about 2 h; then, definitive slices, not more than 5 mm in thickness, were cut with a sharp razor blade. The slices were put in cassettes for further fixation in the same solution (for up to 24 h). The samples were then processed through a rising ethanol series; after butanol, they were infiltrated with and embedded in paraffin wax and sectioned at a thickness of 7 μm . All of the staining methods were according to Bancroft and Cook (1984), but the Mowry's Alcian blue with periodic acid-Schiff (PAS) was that of Pearse (1968) (Table 1). Counterstaining in Fe-demonstration was made with Kernechtrot, and in combination with aldehyde fuchsin, Halami's counterstaining was used (Bancroft and Cook 1984).

X-ray microanalyses were made (with a Zeiss Digital Scanning Microscope 962 equipped with an EDS detector) from carbon-coated (Balzers CED 010 carbon evaporation device) cut or broken sides of frozen, air-dried tissue pieces, except for glochidia, which were preserved in 80% ethanol before the drying and X-ray analysis. The excitation voltage was 20 kV. SE figures were photographed with a JEOL JSEM-820 scanning electron microscope from carbon- or gold-coated (JEOL FINE COAT JFC-1100 sputtering device) air-dried or critical point-dried (Balzers CPD 020 critical point dryer) samples. The SE figures do not necessarily show the exact sites of the element analyses. For comparison, ethanol-preserved or freeze-dried gravid marsupial gill pieces and glochidia of lake mussels, *A. anatina* and *A. cygnea*, were used. The lake mussels were removed from the bottom of Lake Lippajärvi, southern Finland, at the end of October 1993. The demonstration of iron in histological sections of *A. anatina* was made as described above.

RESULTS

Histochemical Analyses

Calcium-Containing Concretions

Staining with alizarin red S (Table 1) showed that the mantle (Fig. 1a) contained many calcified spherules in small fields of different spherule sizes (0.5–2.0 μm) (Fig. 1b). Along the gill axes and above the pericardial glands (Fig. 1a), there were large areas consisting of small and larger (simple or combined) calcified spherules (Fig. 1c). Some spherules occurred even between the

heart muscle cells. Very small granules (0.5 μm) were present among the body wall muscles and between the Leydig cells around the gonad follicles and digestive tubules (Fig. 1a). The calcified spherules and granules also contained Fe^{3+} and sometimes some Fe^{2+} (Table 1; Fig. 1d). The iron was present even after treatment of the sections with an acid fixative (Bouin's), whereas most of the calcium had been lost, unmasking a pink or lilac color, which may be due to the iron and other metals.

In the gills, calcium was detected in the supporting rods and in smaller or even very large simple or combined spherules situated mainly in the interlamellar junctions (Fig. 1a). The calcium spherules in the gills, as well as the gill rods, also contained Fe^{3+} (Fig. 1e) and sometimes some Fe^{2+} (Table 1). The ground substance of the spherules and the gill rods was usually alcianophilic and aldehyde fuchsin-positive. They were sometimes also PAS-positive.

Glochidial Shells

According to the histochemical studies, the glochidial shells of *M. margaritifera* contained calcium but no Fe^{3+} could be detected (Fig. 1e). The ground substance was aldehyde fuchsin-positive, implying the presence of sulfur (Fig. 1f). In the intramarsupial glochidial shells of *A. anatina*, no Fe^{3+} could be detected.

Lipofuscin-Type Concretions

The pericardial gland cells included prominent concretions, which were oval or round, 10–15 μm in diameter (Fig. 2a). The concretions, which were not always homogeneous, may grow within the cells by the union of smaller droplets; finally, the solidified concretion filled the cell almost totally, forcing the flattened nucleus to the base of the cell. Free concretions were also seen in the pericardial cavity. The concretions contained lipofuscin (Fig. 2b), Fe^{3+} , and sometimes a little Ca^{2+} (Table 1). The PAS-positivity may also be indicative of lipofuscin. The MnO_4 oxidation of the concretions turned them aldehyde fuchsin-positive (Figs. 2c–d).

Lipofuscin- (or melanin-) and Fe^{3+} -containing concretions were also detected in the kidney epithelium (Fig. 2e) and in the gonads, particularly in the testes (Table 1). In the testes, most of the concretions occurred freely in the follicles (Fig. 2f), and in the

TABLE 1.
Histochemistry of tissue concretions and glochidial shells in *M. margaritifera*.

Main Sub- stance To Be Demonstrated	Calcium Spherules and Granules	Gill Rods	Glochi- dial Shells	Pericardial Concre- tions	Kidney Concre- tions	Testis Concre- tions	Staining Method	Fixa- tive
Ca^{2+}	2	1–2	1–2	*–1	*	*	Alizarin red S	a,b,c
Fe^{3+}	1–2	1–2	*	1–2	*–2	*–1	Perl's technique	a,b,c
Fe^{2+}	*–1	*–1	*	*	*	*	Schmelzer test	a,b,c
Lipofuscin, melanin	*	*	*	1–2	*–2	*–2	Schmorl's test	a,b,c
Lipofuscin	*	*	*	*–2	*	*–1	Thionin pH 3	a,b
Polysaccharides etc.	*–1	*–1	*–1	1–2	*–1	*–2	Cellobidin-PAS	c
Polysaccharides other than glycogen	*–1	*–1	*–1	1–2	*–1	*–2	Diastase or saliva-PAS	c
Acid mucins	*–2	1–2	*–1	*	*	*	Alcian blue	a,b,c
Organic bound S	*–2	1–2	1–2	*	*	*	Aldehyde fuchsin	b,c
Organic bound S	1–2	1–2	1–2	*–2	*–2	*–2	KMnO_4 -ald.f.	b,c

* not detected; 1, moderate; 2, rich. Fixatives: a, neutral glutaraldehyde-formaldehyde (1G4F, Howard and Smith 1983); b, Lillie's buffered formalin; c, Bouin's fluid.

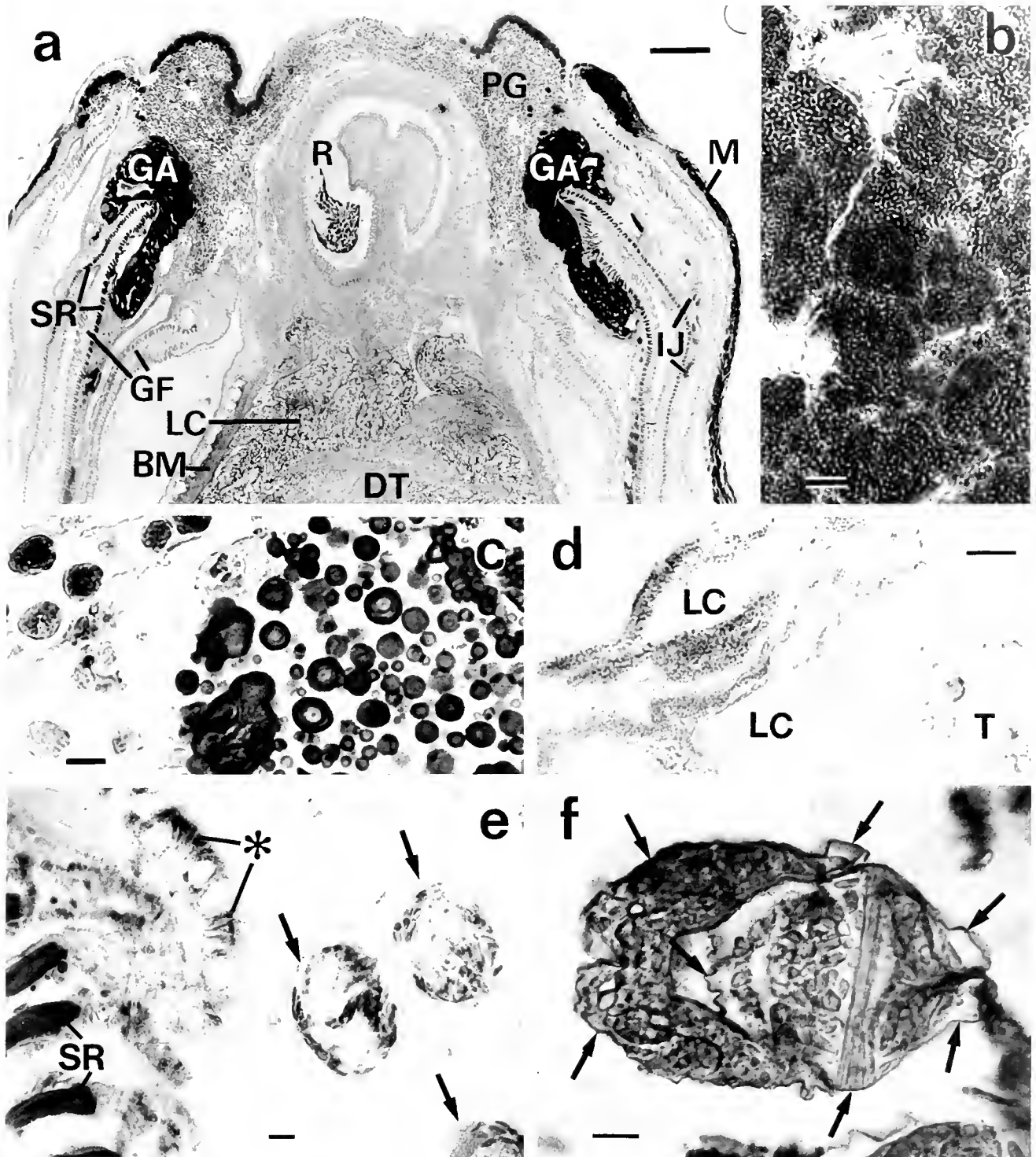


Figure 1. Histochemistry of calcified concretions and glochidial shells of *M. margaritifera*. (a) Dorsal part of a transverse mussel section. Calcium was detected by alizarin red S (dark in this figure, no counterstain) in spherules of the mantle (M), of the gill axes (GA), and of the interlamellar junctions (I.J) of the gills and in supporting rods (SR) of the gill filaments (GF). It was also found in minute granules in the connective tissue (between Leydig cells [LC]) and among muscle fibers (BM) of the body wall. In the pericardial gland cells (PG), it was scarce. DT, digestive tubules; R, rectum. (b) Greater magnification from a mantle stained with alizarin red S. The mantle contains calcium granules of different sizes as separate small fields. (c) Fe^{3+} spherules at the gill axis (to the right) and in pericardial concretions (to the left). Kernechtrot counterstaining. (d) Fe^{2+} in calcified granules between Leydig cells (LC) (no counterstain). T, testis follicle. (e) In the gill, Fe^{3+} is apparent in the cross-sectioned supporting rods (SR) of gill filaments and in some epithelial cells with lipofuscin(?) (asterisk) but not in the glochidial shells (arrows). The shells are wrinkled as the result of decalcification in Bouin's fixative. Kernechtrot counterstaining. (f) Two glochidia (one within the other) stained with aldehyde fuchsin without preoxidation. Halami's counterstaining. The glochidial shells (arrows) show positive staining (purple-violet) for sulfur. Bars, 10 μm or 1 mm (Panel a).

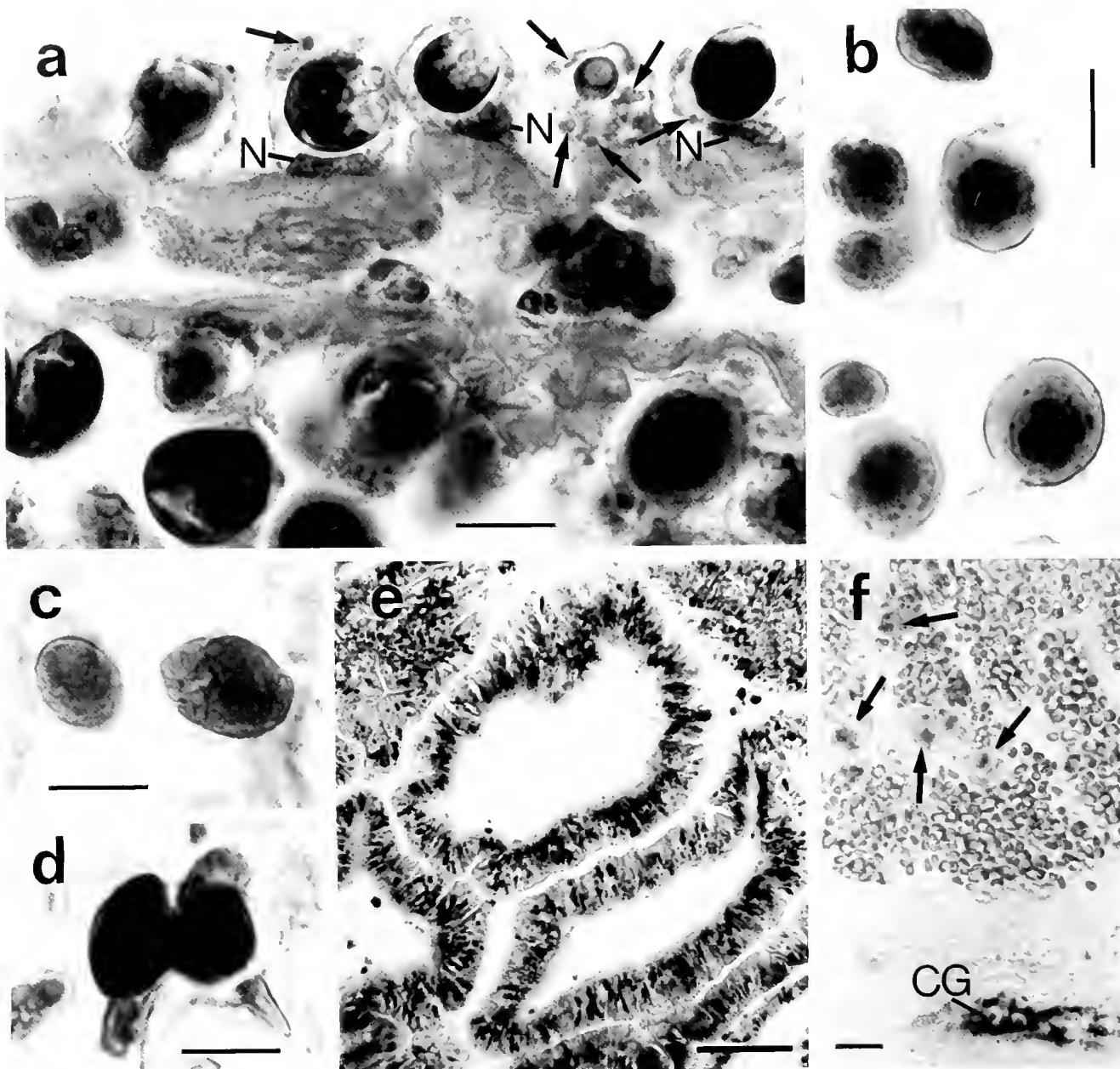


Figure 2. Lipofuscin-type concretions in *M. margaritifera*. (a) Pericardial gland stained with alcian blue-PAS-hematoxylin. The small droplets (arrows) and the large concretions in the gland cells are PAS-positive. N, nucleus. (b) The inner parts of the pericardial concretions stain with thionin at pH 3, indicating the presence of lipofuscin. (c and d) Pericardial concretions are aldehyde fuchsin-negative (yellow-brown) without preoxidation (c) and positive after the KMnO_4 preoxidation (d). (e) Lipofuscin- or melanin-containing granules in kidney tubule cells (Schmorl's method). (f) Concretions in a testis follicle showing the presence of Fe^{3+} (arrows). Calcified granules (CG) outside the follicle stain darkly by Perl's technique. Bars, 10 μm or 100 μm (Panel e).

ovaria, follicle cells contained some concretions. Similar but smaller granules were also detected in the gonoduct epithelium, and very small granules occurred in certain epithelial cells of the gill (Fig. 1e) and of the inner mantle surface. Amoebocytes generally contained lipofuscin and even Fe^{3+} .

X-Ray Studies

Calcified Concretions and Shells

The X-ray analysis spectra from a field of several spherules in the mantle (Fig. 3a) and from a single spherule did not greatly

differ from each other. In both spectra, calcium and phosphorus were the main elements. In the spherules of the gill axis, the proportion of iron to manganese was smaller than in the mantle spherules.

In the gills of *A. anatina*, a species used for comparison, both spherules and rods were analyzed. Their X-ray spectra were identical (that of a rod is shown in Fig. 3b). A spherule in the gill of *A. cygnea* and a gill rod of *M. margaritifera* also showed nearly the same proportions of phosphorus, sulfur, calcium, manganese, and iron (the spectrum of the *M. margaritifera* rod is shown in Fig. 3c) as the spherules and rods of the *A. anatina* gill. In all of these gill

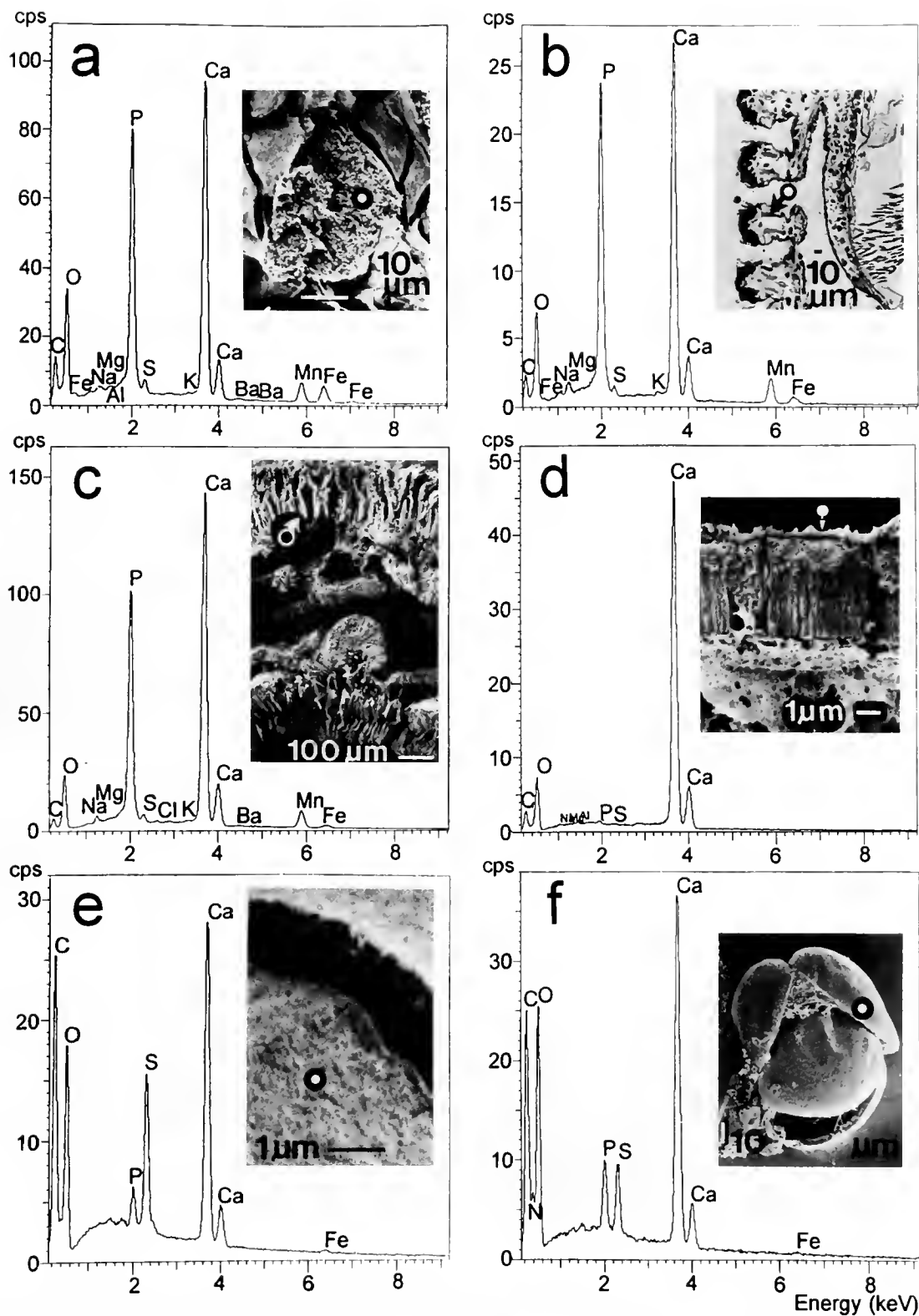


Figure 3. X-ray spectra and SE figures (except for inset in Panel b, which shows a paraffin section) of calcified concretions and glochidial shells of *M. margaritifera* and *A. anatina*. (a) Calcium spherule field in mantle of *M. margaritifera*. (b) A supporting gill rod of *A. anatina*. (c) A supporting gill rod of *M. margaritifera*. (d) Glochidial shell of *A. anatina*. The curve on the fractured surface shows the thickness of the shell. The electron beam was directed through the thin periostracum above the calcified layer. Note the thinness of the shell. (e) Central part of the *M. margaritifera* glochidial shell. (f) Margin of the *M. margaritifera* glochidial shell.

concretions, iron was expressed in a lesser proportion to manganese as compared with the mantle spherules of *M. margaritifera*.

In the glochidial shells of *A. anatina* (Fig. 3d) and *A. cygnea* (not shown), phosphorus (and maybe sulfur) is just detectable and manganese and iron are lacking or below the detection limit. In the spectra of the *M. margaritifera* glochidial shells (Figs. 3e-f), phosphorus and sulfur are detected in varying amounts. Manganese cannot be detected, but iron may be on the border of detection.

In the calcified layers (prismatic and nacreous layers) of the adult *M. margaritifera* shell, phosphorus, sulfur, and calcium were expressed in proportions similar to those in the glochidial shells of *A. anatina* (cf. Fig. 3d with Figs. 4a-b). The mostly proteinaceous periostracum contained very little calcium, but the precipitate on the shell showed a high content of manganese, some silica, calcium, and iron (Figs. 4c-d). The aluminium found in the spectrum may be true or may originate from the stub.

Lipofuscin-Type Concretions

In the pericardial concretions, sulfur was the most prominent element; calcium and iron were apparently present; and phosphorus, chlorine, potassium, and manganese occurred in smaller amounts (Fig. 5a). In the kidney cells, concretions are small, and so, the elements of the cell around the concretions are also greatly expressed (Fig. 5b). Although an orange color specific for calcium could not clearly be separated from the natural brownish color of the kidney concretions by the histochemical method, calcium was found in the X-ray spectrum. Iron was detected by both methods.

DISCUSSION

Calcified Concretions and Shells

M. margaritifera differs from *Anodonta* and *Unio* species in that it has exceptionally rich deposits of calcified spherules in the

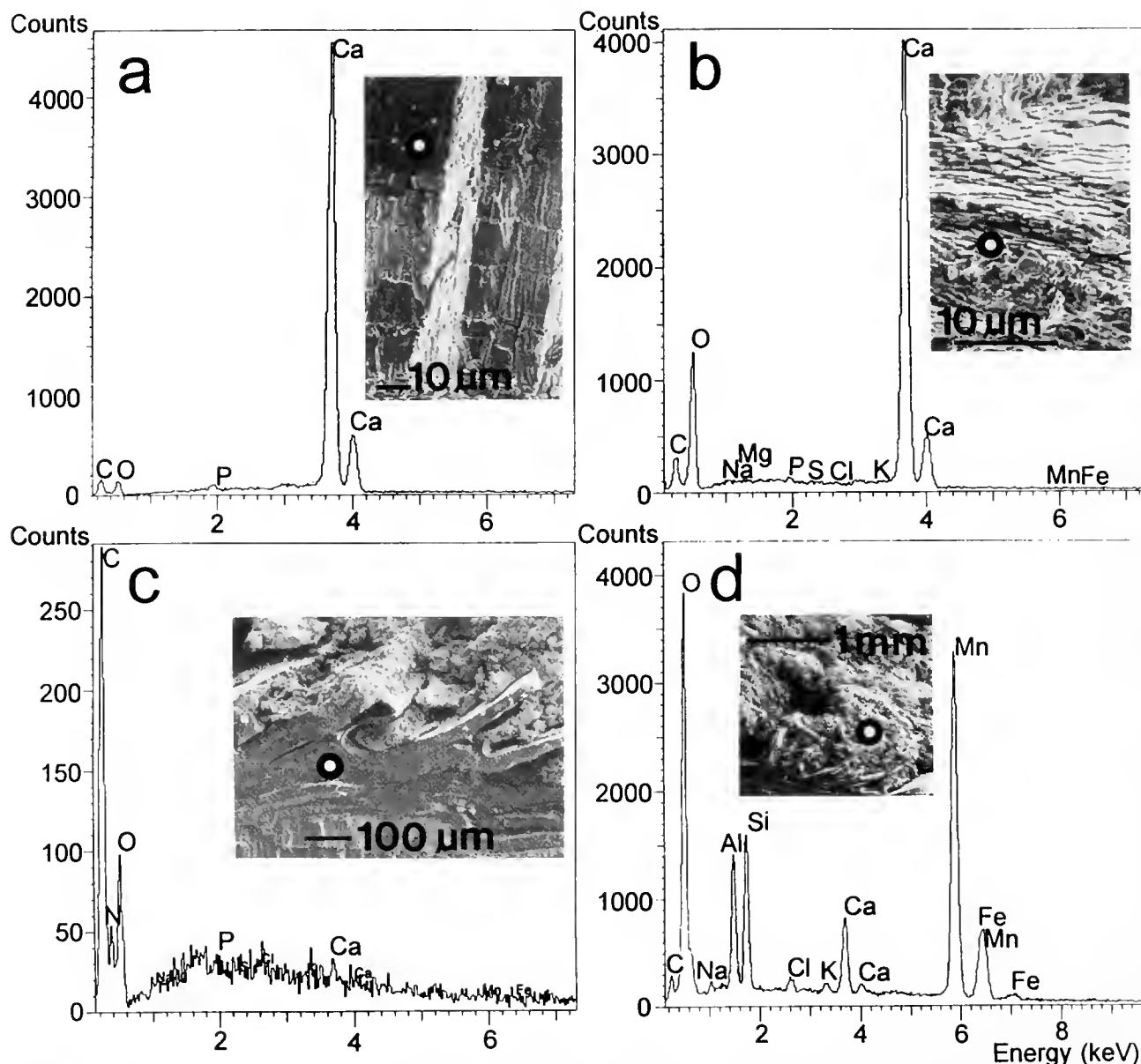


Figure 4. X-ray spectra and SE figures from the shell of an adult *M. margaritifera*. The shell is over 10 cm long, and the cracks are about 1 cm from the edge of the shell. (a) Prismatic layer. (b) Nacreous layer. (c) Periostracum. (d) Precipitate on the shell.

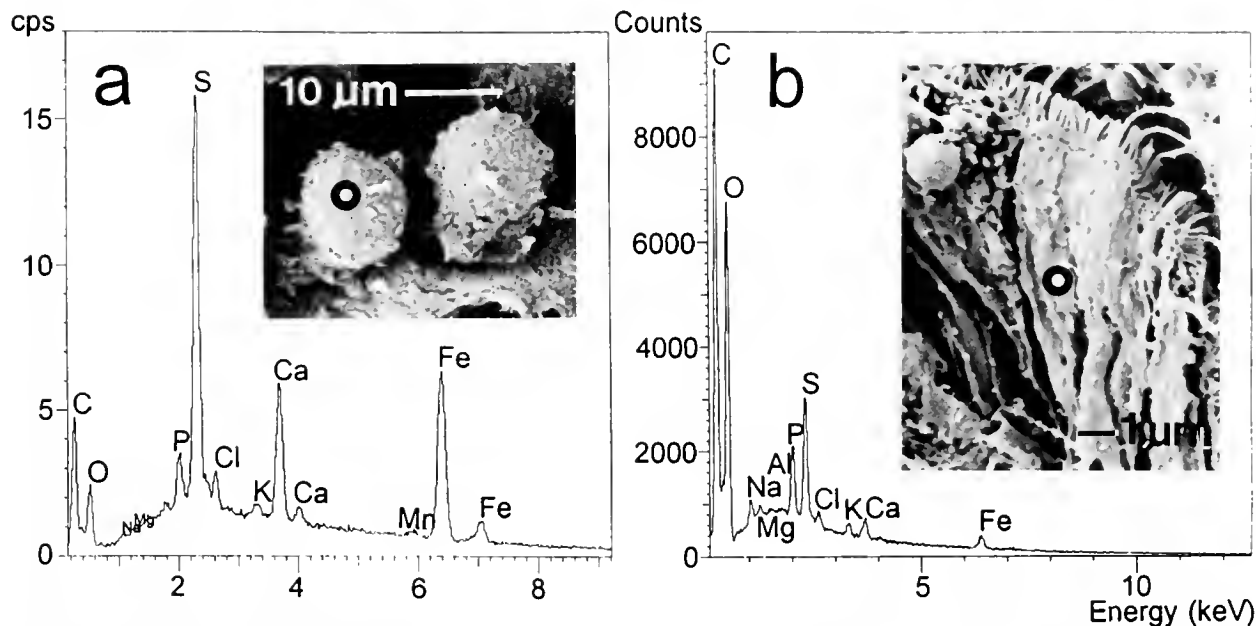


Figure 5. X-ray spectra and SE figures from the pericardial gland (a single cell with concretion) (a) and from kidney cells containing concretions of *M. margaritifera* (b).

mantle (cf. Pynnönen et al. 1987, Pynnönen 1990). The rich calcium depot in the mantle suggests that *M. margaritifera* effectively absorbs calcium, even from very soft water. Although the calcium spherule content was exceptionally high in the mantle of *M. margaritifera*, it was not so in the gills (cf. Fig. 1a with figures in Silverman et al. 1983, Silverman et al. 1985, Silverman et al. 1987a, Pynnönen et al. 1987). This is consistent with the gill concretions of *Margaritifera hembeli* (Conrad) in the United States (Steffens et al. 1985). Calcium from the gill concretions of female mussels is used for the formation of the glochidial shells (Silverman et al. 1985, Silverman et al. 1987a). Although females of *Margaritifera* species produce glochidial larvae in greater numbers than *Anodonta* species (Bauer 1994), the larvae are small and thin shelled (Pekkarinen and Valovirta 1996) and thus do not incorporate very much calcium during their early shell formation.

The results of the staining of the *M. margaritifera*-calcified concretions with alcian blue and the X-ray studies of this work agreed with the previous observations with unionid species: in the calcified concretions, calcium is bound to inorganic or organic phosphates (Silverman et al. 1983, Silverman et al. 1987b, Pynnönen et al. 1987, Lautié et al. 1988). Alcian blue at pH 2.5 stains polyanions with sulfate and carboxyl radicals, but it may, in connection with insoluble calcium salts, also be linked to protein-bound phosphate radicals (Bancroft and Cook 1984). Some of the acidic groups in the ground substance of the calcified concretions of *M. margaritifera* may be sulfate radicals because of the positive aldehyde fuchsin staining. Sulfated glycosaminoglycans may have a role in the transport and storage of calcium, as has been suggested on the gill surfaces of *A. anatina* (Hovingh and Linker 1993).

In bivalve fluids, a bicarbonate- CO_2 buffer system is operating (Byrne et al. 1991). Because in bivalve shells calcium is in the form of carbonate (Rosenberg 1980), calcium carbonate from the shell could naturally be the source of the ions needed. The calcified concretions in unionacean tissues have also sometimes been thought to be the source of the buffering ions (Machado et al.

1988). Only a small portion of the calcium in bivalve mantle is, however, easily interchangeable with the intercellular fluid (Istin and Maetz 1964). It is possible that in the unionacean concretions, there is a small quantity of calcium carbonate, which is more easily solubilized than the phosphates. The prominent concentration of calcified spherules on the gill axis of unionid mussels may ensure the normal functioning of the gill nerves by buffering the hemolymph pH near the main gill nerves (Silverman et al. 1983). Although the concretions in unionid gills are mainly calcium phosphate, calcium is, however, during reproduction, rapidly mobilized for glochidial shell formation (Silverman et al. 1985). The mechanism for this is not known. The possible role of the extensive mantle calcium reserves in the pH regulation of *M. margaritifera* during long-term acid exposures should be studied, although it was not important in unionids (Silverman et al. 1983). The adult freshwater pearl mussel can withstand continuous exposure to pH 5 (Grundelius, according to Carell et al. 1987). During acid stress, the mussel, however, leaks calcium in poorly mineralized water because of intermittent valve gaping (Heming et al. 1988). As a benefit, the gaping allows the exit of some CO_2 . Thus, the mussel is able to maintain a less acidic environment in the mantle cavity fluid than in the surrounding water. It would be very interesting to study the amounts of calcium-containing concretions in the tissues of *M. margaritifera* living in harder waters, or to compare them with those in *M. auricularia*.

Calcium deposition to the shell is regulated through the secretion of the organic matrix and by controlling the hemolymph calcium concentration, the transepithelial electric potential difference in the mantle, the pH of the fluids, and the CO_2 partial pressure in the extrapallial space (Coimbra et al. 1993). *M. margaritifera* lives long and has a fairly thick shell, which apparently accumulates much calcium in its formation. The transfer of calcium to the glochidial shell may be a process similar to that for the adult shell. In both processes, the type of salt has to be changed from phosphate to carbonate. The *M. margaritifera* glochidial shells showed a positive reaction with aldehyde fuchsin. Sulfate groups, among

others, may be involved in the initiation of bivalve shell mineralization (Rosenberg 1980). Some phosphorus and sulfur found in the glochidial shells of *M. margaritifera*, in contrast to the shells of the *Anodonta* species, may result from the mucus secreted by the marsupial gill, from the glochidial mantle, or from a greater proportion of organic matter to calcified spicules in the shell (Pekkarinen and Valovirta 1996). The shells easily wrinkled or collapsed in the vacuum used for the X-ray analysis.

In the *M. margaritifera* tissue concretions, there were also other metals, namely, manganese and iron. This is consistent with the results of Silverman et al. (1983), Pynnönen et al. (1987), and Lautié et al. (1988). In animal tissues, iron is generally trivalent, but in the concretions of *M. margaritifera*, there were some indications of Fe^{2+} . Fe^{2+} has previously also been found in the calcium granules of *A. cygnea*, and it has been thought that symbiotic bacteria in its tissues cause the reduction of Fe^{3+} to Fe^{2+} with the oxidation of Mn^{2+} to Mn^{4+} (Lautié et al. 1988). In the tissues of *M. margaritifera* viewed on the light microscope level, we did not find any bacterial symbionts.

This study shows that iron and manganese, which occur in the concretions of the parent mussel, do not, to a large extent, enter the anodontine and *M. margaritifera* glochidial shells. *Anodonta* species can even regulate the elements incorporated in the gill concretions, so that toxic divalent cations are excluded (Silverman et al. 1987b). Manganese and iron from the natural habitats of bivalves commonly deposit on the shells (Allen 1960, Baer 1984, this study). On the *M. margaritifera* shell in Germany, these elements were shown to occur as manganic(IV) and ferric(III) oxide hydrates (Baer 1984). In this study, manganese and iron in the nacreous and prismatic layers of the *M. margaritifera* shell remained below the detection limit, but with more sensitive methods, Carell et al. (1987) could demonstrate even temporal variations in the accumulation of these elements.

Lipofuscin-Type Concretions

Lipofuscin- and often metal-containing (e.g., iron-containing) granules generally occur in bivalve kidneys (George et al. 1982, Nigro et al. 1992). George et al. (1982) found that in the *Mytilus* kidney granules, the major anionic components were those containing phosphorus, sulfur, and chlorine. Sulfur may occur as sulfide or —SH groups (Nigro et al. 1992, Roesijadi 1992). In this study, phosphorus, sulfur, and a small amount of chlorine were detected in the kidney cells of *M. margaritifera*. The lipofuscin-type concretions in the gonads of *M. margaritifera* may be an indication of resorption, lysis, and excretion of undeveloped and atretic germinal cells.

The ultrafiltration of urine takes place in the pericardial glands (Andrews and Jennings 1993, Meyhöfer and Morse 1996). The glands may accumulate soluble foreign material and have the potential to function in detoxification and degradative processes (Zarogian and Yevich 1993). Although Fe^{3+} and Ca^{2+} were found in the pericardial gland concretions of *M. margaritifera*, the polyhedral inclusion bodies in large cytoplasmic vacuoles of the epithelial cells of the gland in the mussel *Bathymodiolus thermophilus* did not contain metals but were possibly made of proteins (Auffret and LePennec 1992). The concretions in *M. margaritifera* may be a means for the concentration and excretion of waste products of lipid and protein metabolism and the metals bound to them.

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POTENTIAL FOR POPULATION REGULATION OF THE ZEBRA MUSSEL BY FINFISH AND THE BLUE CRAB IN NORTH AMERICAN ESTUARIES

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ABSTRACT We conducted a series of descriptive and manipulative experiments aimed at quantifying the abundance, natural mortality, and effectiveness of predators in controlling the zebra mussel, *Dreissena polymorpha*, in the Hudson River Estuary. First, we measured distribution, abundance, and mortality rates of a zebra mussel population in the middle portion of the Hudson River Estuary, NY. Rocks were collected along a depth gradient in the field and sampled for the density and size structure of the resident mussels over the growth season. Next, we either allowed access (controls) or denied access (predator exclusion) to predators in field experiments with rocks harboring a known number of zebra mussels to estimate natural mortality. Finally, we conducted manipulative field experiments to test the effectiveness of the blue crab, *Callinectes sapidus*, at consuming zebra mussels by presenting similar rocks to crabs in field enclosures. Field sampling in June, July, and August 1993 indicated a dense (~30,000 mussels/m²) population composed of a single cohort of 1 + year-class mussels. Sampling in August 1994 indicated a decline in *D. polymorpha* density. Mussel density increased dramatically with depth less than 2 m below the spring low tide mark. In cage experiments, blue crabs caused mortality rates that were an order of magnitude higher than those measured for the local predator guild, which was primarily composed of finfish. Localized extinctions of zebra mussels within one growth season were predicted in areas where blue crab densities approach 0.1 crabs/m².

KEY WORDS: Zebra mussel, blue crab, population regulation, predation

INTRODUCTION

Predation can regulate community structure and the dynamics of marine benthic species (Peterson 1979, Paine 1980). Predator-prey interactions in marine systems are particularly complex and may be relatively stable because they are dominated by guilds of generalist predators capable of switching among numerous prey species (Peterson 1979, Hines et al. 1990). The abundances of such generalist predators are not coupled to their benthic prey and, therefore, are capable of controlling the dynamics of these prey species or driving them to local extinction without being dependent on any single species for their persistence. (Murdoch et al. 1985). Generalist predators have long been cited as regulators of population structure in the classic studies of the marine intertidal zone (Connell 1970, Paine 1974). In this setting, a successful predator may prevent the establishment of or destroy monoculture of a competitively dominant species (Paine 1992). The varied nature of the predator's diet is necessary for it to persist during periods of low abundance of the dominant prey species. Such features potentially characterize predator-prey interactions between the exotic zebra mussel, *Dreissena polymorpha* (Pallas), and natural predators such as the blue crab, *Callinectes sapidus* (Rathbun), and thereby provide the requisite conditions for predator-mediated control of *D. polymorpha* population dynamics.

The zebra mussel was first discovered in the Hudson River in 1991 and has since expanded to its salinity limit (3–6 ppt) near Haverstraw, NY (Strayer et al. 1993). The rapid colonization of North American waters has been facilitated by its high fecundity (30,000 eggs/female per year), a free-swimming larval stage that is unlike that of any native freshwater bivalve, and the apparent lack

of effective competitors and predators (Hebert et al. 1991, Lemma et al. 1991, MacIsaac et al. 1991, Strayer 1991). As a consequence, *D. polymorpha* often occurs at densities exceeding 10,000 mussels/m² and has thereby become a major and costly nuisance (Cooley 1991, Griffiths et al. 1991). Zebra mussels attached to hard substrates by their byssal fibers form large colonies, which can choke off water intake pipes at power plants and municipal water treatment plants and also produce biofouling problems on boats, navigational aids, and beaches.

Moreover, as a result of its salinity tolerance (up to approximately 5 ppt), the zebra mussel is expected to colonize and expand into most North American waters, including the low-salinity portions of estuaries such as Chesapeake Bay (Bij de Vaate 1991, Strayer 1991, Strayer and Smith 1993). Thus, the potential exists for *D. polymorpha* to become a serious pest throughout its environmentally delineated range in North American waters, unless predation or competition can effectively regulate the zebra mussel in its distribution and abundance.

The blue crab is a large (males up to 227-mm carapace width [CW]), epibenthic omnivore occurring in various habitats along the northwest Atlantic Ocean, Gulf of Mexico, and Caribbean Sea (Williams 1984). Blue crabs serve as both prey and consumers and are abundant and actively forage from late spring through autumn in Chesapeake Bay (Hines et al. 1987, Hines et al. 1990). The diet of Chesapeake Bay blue crabs consists of bivalves, crabs (both blue crabs and xanthids), fish, and polychaetes, and to a lesser extent, amphipods and isopods (Hines et al. 1990, Mansour and Lipcius 1991). Blue crab ecology in the Hudson River has not been well studied, and consequently, the abundance and range of the species within the system are not understood. Previous research has shown that *C. sapidus* is common in the freshwater and low-salinity regions of the estuary in some years (Stein and Wilson 1992). Strayer et al. (1993) reported that blue crabs in the Hudson River included zebra mussels in their diet. Laboratory experiments demonstrated that adult male blue crabs readily consumed zebra

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mussels and preferred the largest individuals available (Molloy et al. 1994).

In this investigation, we quantified abundance patterns and natural mortality rates of *D. polymorpha* in the field and tested the hypothesis that predation by *C. sapidus* and naturally occurring finfish predators might serve to limit the zebra mussel in the Hudson River Estuary and in other North American estuaries. We conducted quantitative sampling and a series of field experiments in Hudson River freshwater habitats to determine limitations imposed by finfish and the blue crab on zebra mussel abundance and distribution. Further trials compared the effectiveness of the blue crab and the local predator guild (primarily, finfish species) in controlling zebra mussel abundance. The specific objectives of the investigation included: (1) a description of *D. polymorpha* abundance and distribution, (2) measurement of natural mortality of *D. polymorpha* and identification of likely predators, and (3) testing the feasibility of biological control of *D. polymorpha* by *C. sapidus* and finfish in the Hudson River.

METHODS

Study Site

We conducted field experiments and collected samples on the eastern shore of the Hudson River in the Tivoli Bays Region of the Hudson River National Estuarine Research Reserve, NY (42°05'N, 73°55'W) (Fig. 1). The tidal freshwater habitat was approximately 160 km north of the mouth of the estuary. In this region, the benthic environment of the Hudson was characterized by large stones and cobbles covering a steeply sloping bottom that reached over 20-m depths in some areas. The tidal range was approximately 1.0 m, and underwater visibility was poor (<3 m) during the study periods because of suspended particles.

Zebra Mussel Sampling

In the first component of this study, rocks were sampled by SCUBA divers during June, July, and August 1993, and again in

August 1994, to examine the density and size structure of the zebra mussel population. Divers collected rocks haphazardly by hand at depths ranging from 3 to 20 m during the four sampling periods. Rocks with attached mussels were transported to the laboratory in padded coolers to minimize handling mortality. We estimated zebra mussel density on each rock by removing all live individuals that fell within a 16-cm² plastic grid placed on the rock's surface. Mussels were removed by pulling the byssal fibers from the substrate surface with forceps. These mussels were counted, and their shell lengths were measured to the nearest millimeter with Vernier calipers. Six replicate rock samples were examined during each month of the study, yielding 24 samples during the 1-y period. Mean zebra mussel densities were used to estimate both inter-annual and intra-annual mortality rates. Shell length data were used to construct size-frequency distributions.

We conducted a series of five underwater transects in August 1993 to characterize the depth distribution of *D. polymorpha* at the study site. Four random rock samples were collected using SCUBA along depth profiles to determine density using the same method as above. The four samples at each depth were located along a marked transect line that was positioned by divers. A random number table was used to select the four marks along the line at which a rock would be taken. Densities reflect the average number of animals per area of rock surface, not area of river bottom. At each collection site, a visual estimate of percent coverage was also taken with a haphazardly placed circular grid (25 cm in diameter). Samples were collected along a transect at increasing depths (0.5-m increments) until 100% coverage was observed at all four sample locations. Transects were conducted at 0.5-, 1.0-, 1.5-, 2.0-, and 2.5-m depths. These values were corrected to reflect depth below spring low tide levels using published tide tables.

Field Experiments

The second component of the study involved manipulative field experiments conducted in late July and early August 1993. We first

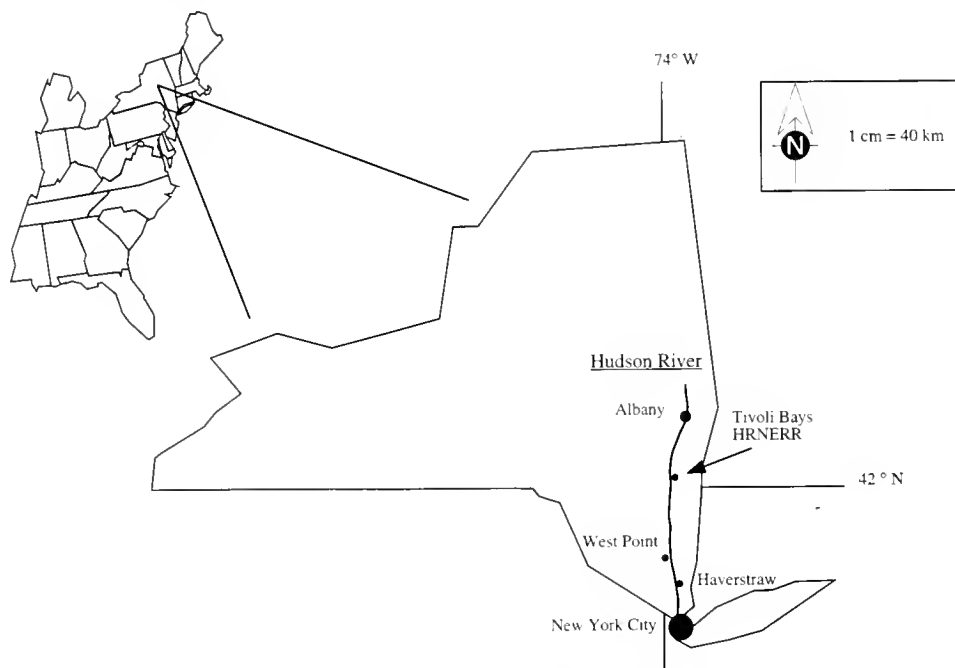


Figure 1. Map of study area in the vicinity of the Hudson River National Estuarine Research Reserve (HRNERR).

measured mortality rates of *D. polymorpha* due to predation. Rocks with attached mussels were collected from the Hudson River by divers and maintained in laboratory aquaria for 72 h to ensure the health of experimental animals. Zebra mussels that actively siphoned water and closed their shells when agitated were considered healthy. After this observation period, mussels were removed from aquaria and placed in dissecting trays. We then began removing mussels from the rock's surface until only 100 live zebra mussels remained attached. Mussels were first removed from the outside surfaces of each rock so that each clump of 100 mussels resembled a naturally occurring cluster. Sixteen of these rocks with 100 attached mussels were then transported back to the field and placed in enclosures for the experiment. Cages were constructed of 2.5-cm plastic mesh, covered 1 m² of substrate, and were 0.7 m tall. Sixteen cages were arranged in four rows of four cages, with 1 m spacing between each, and treatments were interspersed (Fig. 2). Each treatment was replicated eight times. Control treatments comprised fully enclosed cages protecting one rock with 100 precounted mussels. Experimental cages were topless, had only two sides, and thus exposed the experimental rock to predation. After 14 days, the rocks were removed from the cages and the surviving mussels were enumerated.

The final experiment used the same field enclosures and another set of rocks with 100 precounted mussels prepared in the same manner. In this trial, 18 interspersed cages were fully enclosed and hard intermolt male blue crabs were introduced as predators (Fig. 3). Six cages contained small crabs (60- to 80-mm CW), and six cages contained large crabs (110- to 130-mm CW). Six cages contained only rocks with 100 precounted mussels and served as controls. After 72 h, crabs were removed and surviving mussels were enumerated. Each blue crab was examined to confirm that it had survived the entire experimental period.

In both field experiments, the proportional mortality of *D. polymorpha* was calculated by subtracting the number of surviving mussels from the original number of mussels and then dividing that result by the original number of mussels. Differences between treatments were analyzed by use of an analysis of variance (ANOVA) model, with arcsine-transformed proportional mortality as the dependent variable and cage treatment as a fixed factor. Scheffe's test was used to examine contrasts among the three treatments in the second field experiment. Data were examined for normality and tested for homogeneity of variance with an F_{\max} test. (Sokal and Rohlf 1980).

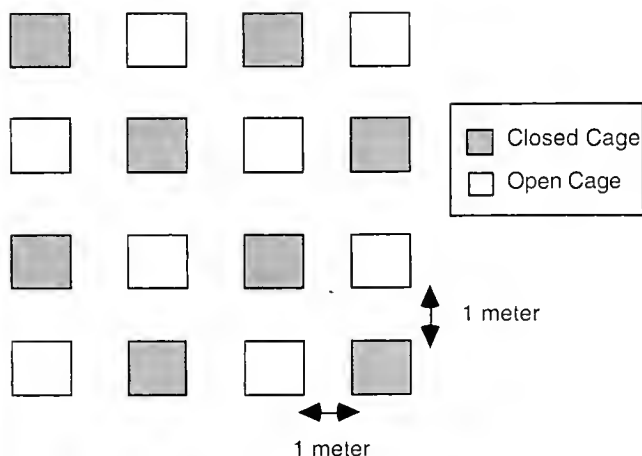


Figure 2. Configuration of cages for the first field experiment.

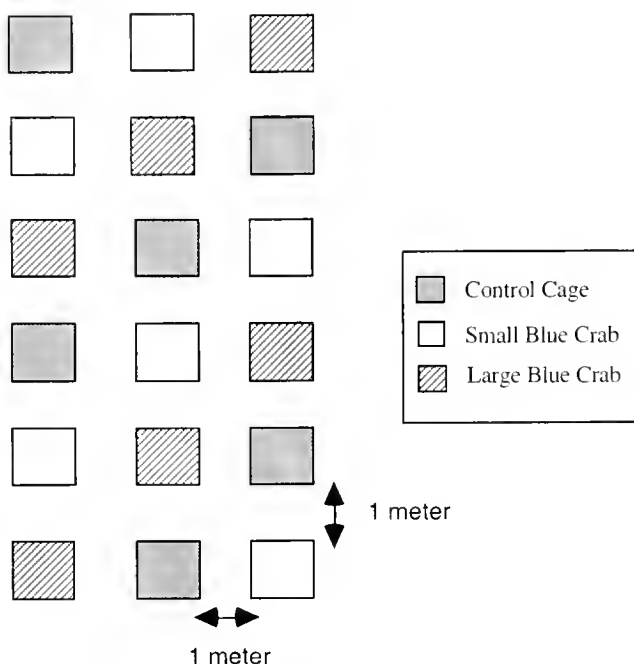


Figure 3. Configuration of cages for the second field experiment.

Instantaneous per capita mortality rates (z) were calculated for each period during the study using the estimated zebra mussel densities. The rate was calculated by:

$$z = \frac{-\ln\left(\frac{N_t}{N_o}\right)}{t}$$

where the instantaneous rate (z) takes into account the original number of mussels (N_o) and the number of mussels (N_t) surviving some period of time (t). This rate (z) was also used to compare zebra mussel mortality rates from the two caging experiments.

Identification of Potential Predators

We recorded over 8 h of underwater video using a Sony 8-mm video recorder with remote waterproof cameras in August 1994. The remote camera was anchored to the rocky substrate using large concrete bricks and pointed at rocks covered with zebra mussels. Poor underwater visibility limited the camera's field of view to approximately 1 m in all directions but did allow it to capture images of fish swimming along the river's bottom. Whenever possible, we identified these fish to the lowest possible taxonomic level.

Six baited crab pots were also fished near the study site during periods of sampling and field experimentation (June, July, and August 1993 and August 1994). These were checked daily for the presence of blue crabs and rebaited when necessary.

RESULTS

The abundance of zebra mussels rapidly increased with increasing depth and reached constant values less than 2 m below the surface. Samples collected along depth transects beginning at the spring low tide mark indicated a significant effect of depth (Fig. 4; ANOVA, $F = 13.88$ $df = 4,15$, $p < 0.0001$). Abundance at the shallowest depth (0.26 m) was significantly lower than at the four deeper stations (Scheffe's test, critical value = 1.329, $p < 0.05$).

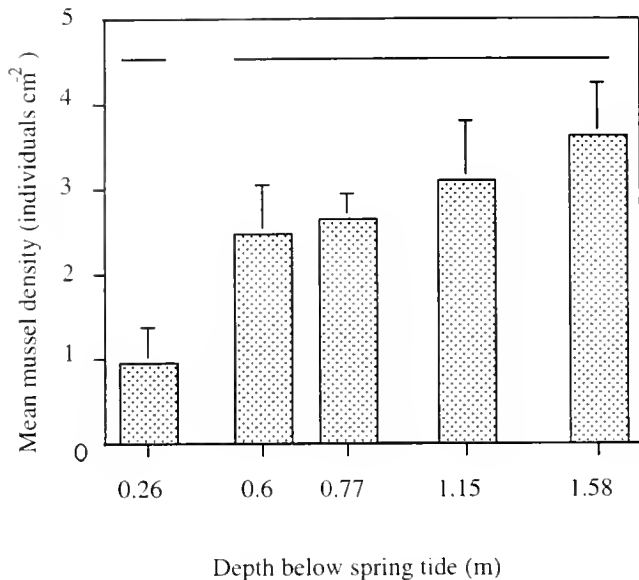


Figure 4. Depth distribution of mean *D. polymorpha* density in the Tivoli Bays region of the Hudson River. Lines connect bars that are not significantly different.

and appeared to reach an asymptote in density at 0.6 to 1.6-m depths (Fig. 4). Density values observed at the 1.6-m transect were similar to those observed at deeper depths during subsequent sampling.

Size-frequency distributions from 1993 (Fig. 5) revealed a single cohort with no individuals exceeding 20 mm in shell length. Mean shell length increased 24% over the 3-mo period from 9.83 mm in June to 11.51 mm in July, and to 12.19 mm in August. Mean mussel density decreased from 4.40 individuals/cm² in June to 3.69 individuals/cm² in July. Mussel density continued to decrease from 3.69 individuals/cm² in July to 3.04 individuals/cm² in August. The instantaneous mortality rate (τ) of zebra mussels during the June to July period was 0.008/day and decreased to 0.005/day during the July to August period.

Size-frequency distributions (Fig. 6) of zebra mussels sampled from rocks in the Hudson River in August 1994 revealed a trimodal population composed of two year-classes. The first, centered around 5-mm shell length, was composed of mussels that settled either late in the fall of 1993 or early in the summer of 1994. The second group, averaging around 20-mm shell length, most likely settled in 1992. Overall, average mussel density was 1.96 individuals/cm² of rock substrate. This indicated a ~35% decrease in overall zebra mussel abundance during the 12-mo period from August 1993 to August 1994. However, the density estimates from 1993 were based only on the population that was represented here by the 2-y-old class. The average density of that year-class (1.18 individuals/cm²) represents a 61% decrease in zebra mussel abundance.

Field Experiments

Mean zebra mussel mortality in the first manipulative experiment was significantly greater (ANOVA, $F = 13.43$, $df = 1,14$, $p < 0.0026$) in the experimental treatments (Fig. 7). Mussels in the closed-cage controls suffered less than 10% mortality over the 2-wk period. In the open cages, attached *D. polymorpha* experienced 24% mortality. The resulting 14% mortality was attributed

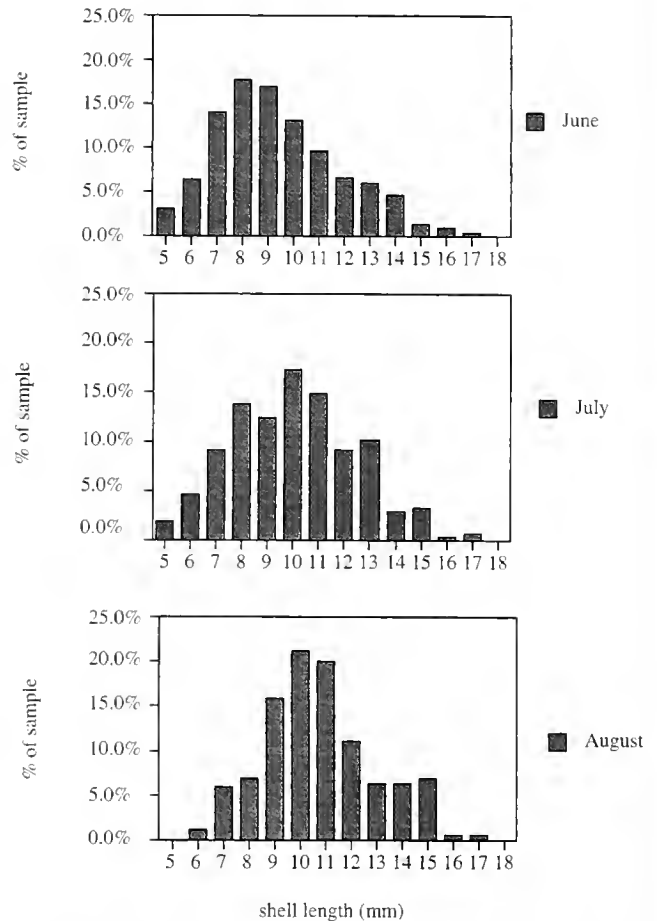


Figure 5. Size-frequency distributions of *D. polymorpha* in the Tivoli Bays region of the Hudson River in 1993.

to the effects of local predators. Zebra mussels in the open cages experienced an instantaneous mortality rate of 0.013/day during the experiment.

The introduction of male blue crabs produced higher mortality rates in the second field experiment. Large blue crabs consumed nearly 40% of the prey in 72-h trials (Fig. 8), correcting for the 10% mortality in the controls during the trial period. The control

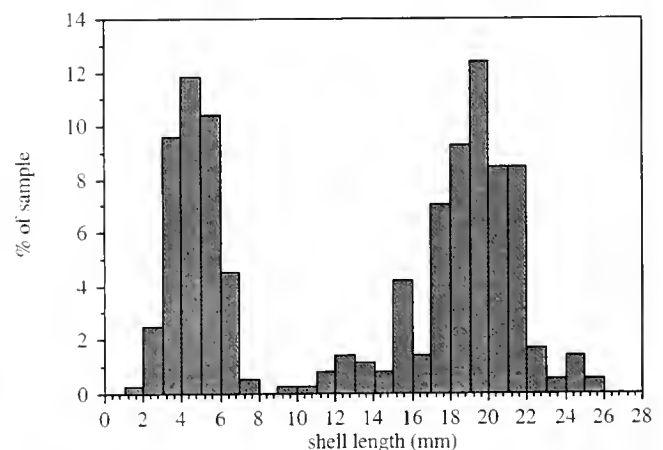


Figure 6. Size-frequency distributions of *D. polymorpha* in the Tivoli Bays region of the Hudson River in 1994.

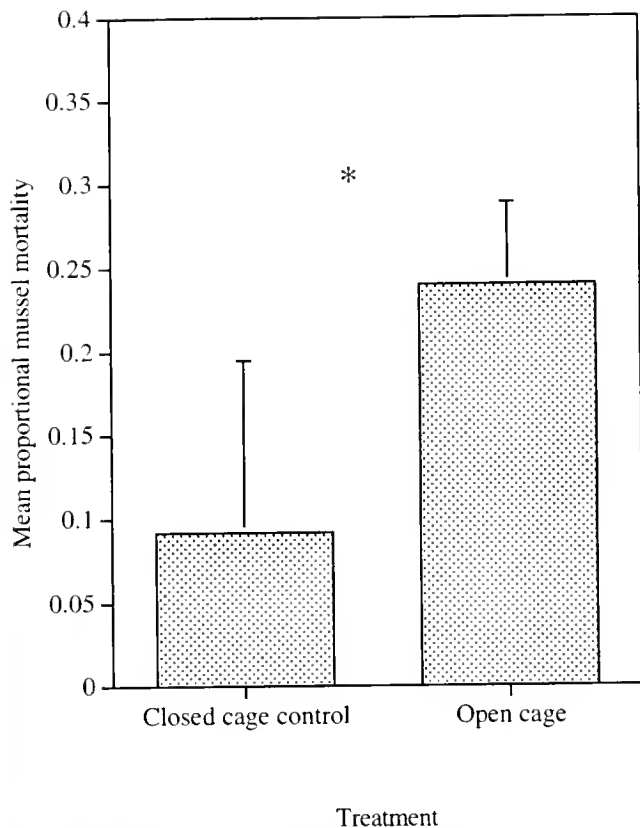


Figure 7. Mean proportional mortality of *D. polymorpha* in open- and closed-cage treatments. Asterisk denotes significant difference.

mortalities in this experiment were similar to those in the first field experiment and were attributed mainly to the handling and transport of mussels between the field and laboratory. Although the effect of the crab treatments was highly significant (ANOVA, $F = 19.21$, $df = 2, 15$, $p < 0.0001$), mussel mortalities did not differ significantly between large and small crab treatments (Scheffe's test, critical value = 0.169, $p > 0.05$). Corrected instantaneous mortality rates (λ) indicated that mortality rates were an order of magnitude higher in those treatments containing blue crabs than in those exposed to natural predators (Table 1).

Potential Predators

Approximately 8 h of 8-mm underwater videotape revealed several fish species occupying the benthic habitat of the Hudson River (Table 2). French (1993) reported that several of these species were capable of consuming bivalves such as zebra mussels. Consumption of mussels by pumpkinseed, *Lepomis gibbosus*, was observed in the video, as well as in the field, by divers on several occasions. Baited crab pots fished during sampling periods in 1993 and 1994 caught no blue crabs. Blue crabs were neither observed during video monitoring nor seen by divers during the study period.

DISCUSSION

The spread of the zebra mussel into the Hudson River Estuary was predicted by Strayer and Smith (1993) and has been well documented. Mussels at the Tivoli site were found at very high densities on hard substrata and were differentially distributed with

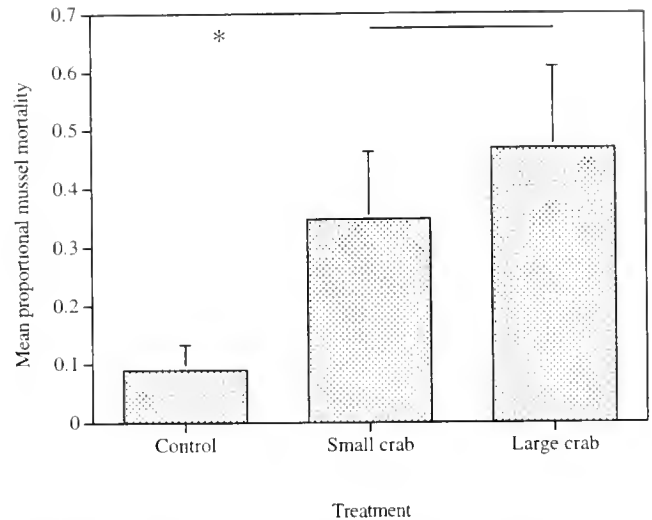


Figure 8. Mean proportional mortality of *D. polymorpha* in control, small crab, and large crab treatments. Asterisk denotes significant difference. Bar denotes nonsignificant difference.

depth. The distribution of increasing mussel density with depth was consistent with the hypothesis that physical factors (e.g., desiccation, ice scour) restrict the upper limit of the vertical abundance of *D. polymorpha* in the Hudson River Estuary. Zebra mussels have been reported in the intertidal region of the St. Lawrence Estuary (Mellina and Rasmussen 1994), but no exposed mussels were observed in this study. Mussels at the shallowest depths (<0.5 m) were most often found in sheltered areas, on the vertical surfaces of rocks or in crevices.

Zebra mussels in European lakes and large rivers occur at densities near 3,000 mussels/m² (Bij de Vaate 1991). The densities reported here ($\sim 30,000$ mussels/m²) are well within the ranges observed in North American waters (Dermott and Munawar 1994). Size-frequency distributions of *D. polymorpha* in the Hudson River indicated that the population was composed of a single cohort spawned the previous year (Jenner and Janssen-Mommen 1993). Given the planktonic larval stage of the mussel, the likely parental population was several kilometers upriver of the Tivoli Bays site (Strayer et al. 1993).

We estimated the natural mortality of zebra mussels from both field sampling and predator-exclusion experiments. In the first case, mussels experienced instantaneous mortality rates of 0.008/day from June to July and 0.005/day from July to August. These estimates were lower than those observed in the predator-exclusion experiment (0.013/day). The higher mortality rates associated

TABLE 1.
Mean zebra mussel mortalities summarized from 1993 field experiments.

Condition	Technique	Instantaneous Mortality Rate/Day
Natural predators	Size-frequency analysis	0.007
	Field experiments, exposed	0.013
Small blue crabs	Predator enclosures	0.119
Large blue crabs	Predator enclosures	0.185

TABLE 2.

Potential piscine predators (based on French 1993) of *D. polymorpha* observed in the Hudson River Estuary by underwater video system.

Common name	Scientific name	Potential Predator	Observed Predation
Pumpkinseed	<i>Lepomis gibbosus</i>	Yes	Yes
Redsunfish	<i>Lepomis auritus</i>	Yes	Yes
Common carp	<i>Cyprinus carpio</i>	Yes	No
Smallmouth bass	<i>Micropterus dolomieu</i>	No	No
Various minnows	Several genera	No	No

within the manipulative experiment suggested some caging effect. Hall et al. (1990) found that although caging treatments can be a powerful research technique, care must be taken in the analysis of results to separate any confounding effects of the method. The presence of a partial cage structure in the experimental treatments may have increased predation rates by attracting more fish.

The success of the zebra mussel in North America can be attributed at least in part to the lack of effective natural predators. In Europe, mussels are preyed on by eels (de Nie 1982), other fish (Daoulas and Economidis 1984), and ducks (Draulans 1984). The role of predation in the recent invasion of North American waters by the zebra mussel is not well documented. At least six species of piscine predators capable of consuming zebra mussels were reported by French (1993) (Table 2), but most of these are uncommon in the Hudson River. Only two of these, the pumpkinseed, *L. gibbosus*, and the red-breasted sunfish, *Lepomis auritus*, were observed consuming *D. polymorpha* during this study. More recently, Hamilton et al. (1994) found that diving ducks in Lake Erie have included zebra mussels in their diet, thus leading to ephemeral reductions in mussel biomass in shallow areas. This study is the first attempt to measure the effects of predation on an estuarine population of *D. polymorpha*.

Predation often functions to control invertebrate species in benthic environments (Virmstein 1977, Brönmark 1988). For exotic species, one of the leading causes of failure to become established in new environments is predation (Lodge 1993). Before the invasion of the zebra mussel, perhaps the most infamous exotic bivalve was the Asiatic clam, *Corbicula fluminea*. Similar to the zebra mussel, this organism led to problems, including biofouling and displacement of native bivalve species. Strong predation pressure by several native fish species limited the success of the Asiatic clam in colonizing at least one potential habitat area (Robinson and Wellborn 1988).

We have suggested that the blue crab might be an effective predator capable of controlling the population dynamics of the zebra mussel. Consumption of *D. polymorpha* by *C. sapidus* was reported soon after the invasion of the Hudson River (Strayer et al. 1993). Molloy et al. (1994) reported a marked reduction of zebra mussels in the mid-Hudson in 1992, which coincided with a high abundance of blue crabs. The observation of high mortality rates in 1992 supported our hypothesis and encouraged our field experiments. The probable characteristics of this predator-prey system that render it amenable to control of *D. polymorpha* by *C. sapidus* include:

- (1) *D. polymorpha* is an epibenthic colonizer of hard, accessible substrates.
- (2) *D. polymorpha* achieves a relatively small adult size, ap-

parently well within the minimum size capabilities of *C. sapidus* predation (Eggleston 1990a, Eggleston 1990b).

- (3) *D. polymorpha* lives in large, discrete aggregates readily apparent to epibenthic predators.
- (4) *C. sapidus* is a generalist predator independent of the densities of any particular prey species (Lipcius and Hines 1986, Hines et al. 1990).
- (5) *C. sapidus* readily consumes bivalves, including mussels (Seed 1980, Blundon and Kennedy 1982, Arnold 1984, Lipcius & Hines 1986, Eggleston 1990a, Eggleston 1990b).
- (6) The functional response of *C. sapidus* to bivalves in habitats providing high encounter rates, such as hard substrates accessible to a predator, is inversely density dependent (Eggleston 1990a, Eggleston 1990b), which leads to localized extinction of the prey (Lipcius and Hines 1986).
- (7) *C. sapidus* aggregates at high-density prey patches (Hines et al. 1990, Mansour and Lipcius 1991).
- (8) *C. sapidus* can tolerate and actively forage in the full range of salinities from marine to freshwater (DeFur et al. 1987).

The results of our crab predation experiment provided support for the hypothesis that dense populations of blue crabs can be more effective in reducing zebra mussel abundance than local finfish or invertebrate predators. *D. polymorpha* mortality rates caused by *C. sapidus* were nearly twice those caused by the local predator guild only 20% of the time. The instantaneous mortality rates (z) observed in the various treatments were used to estimate the time (t) until zebra mussel population levels reached 1% of their current values (Table 3) by the formula:

$$t = \frac{\ln N_0 - \ln N_t}{z}$$

where N_0 is the initial number of mussels and N_t is the number of mussels at the end of the experimental period. Assuming predation by *C. sapidus* would occur over roughly a 100-day period (given the usual absence of blue crabs in the oligohaline portions of estuaries during cooler months), significant reductions of zebra mussels are predicted within one summer (Fig. 9). At our measured predation rates, blue crab densities of 0.1 crabs/m² would drastically reduce the abundance of *D. polymorpha* in one season. A significant decrease in the mussel population would be expected whenever crab densities and predation rates approach or surpass these levels. Hines et al. (1987) reported summer densities of 0.10–0.73 crabs/m² in a subestuary of Chesapeake Bay, MD. During part of the study, water temperature and salinity conditions in the area were similar to those found in the Hudson River Estuary.

Blue crab densities in the Hudson River system are relatively low, varying from almost zero to moderate densities capable of

TABLE 3.

Estimated time to 1% of 1993 zebra mussel abundance based on instantaneous mortality rates observed in field experiments.

Condition	Technique	Estimated Time (t)
Natural predators	Size-frequency analysis	657
Natural predators	Field experiments, exposed	354
Small blue crabs	Predator enclosures	39
Large blue crabs	Predator enclosures	24

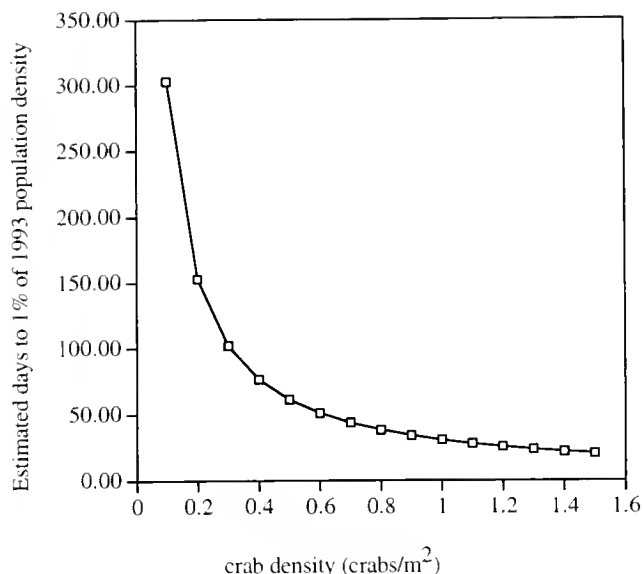


Figure 9. Projected localized extinction rates (days) for *D. polymorpha* at various blue crab densities.

supporting a small commercial fishery in some years (Stein and Wilson 1992). In this study, no crabs were caught in several baited traps, and local fishermen indicated that there were few blue crabs in the middle portion of the Hudson River in 1993 and 1994. Hence, biological control of the zebra mussel in the Hudson River caused by blue crab predation is unlikely.

In conclusion, *D. polymorpha* will not be regulated by the local

predator guild in the Hudson River unless predator abundance increases significantly. This conclusion is supported by the recent estuary-wide investigation by Strayer et al. (1996), which points to competition for food resources as the most important regulatory mechanism in the Hudson River. In particular, the blue crab is capable of controlling zebra mussel abundance if the predator abundance increases to levels approximating 0.1–1.0 crabs/m², depending on crab size. Localized extinctions of zebra mussels within a 100-day growth season, like those observed by Molloy et al. (1994), are possible at these crab densities, given the rates of predation measured in this study (Fig. 9). It is not yet known if blue crab populations reach this level in the Hudson River. Such densities are common in other estuaries, such as Chesapeake Bay, and indicate that the zebra mussel may be regulated in estuaries near the southern limit of its predicted range, where blue crabs are more abundant.

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THE EFFECTS OF *DREISSENA POLYMORPHA* (PALLAS) INVASION ON AQUATIC COMMUNITIES IN EASTERN EUROPE

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ABSTRACT *Dreissena polymorpha* has been invading fresh waterbodies of eastern and western Europe since the beginning of the 19th century and is still invading. A long history of monitoring and experimental studies conducted in the Former Soviet Union (FSU) has provided us with an understanding of the effects of zebra mussels on waterbodies they invade. However, this work has not been generally available. We review work conducted in the FSU and eastern Europe over the past 60 y on the community effects of this invading species. In freshwater areas, where *Dreissena* are the only bivalves that attach to hard substrates and have a planktonic larval stage, they can become enormously abundant and, within a short period of time, can obtain a biomass 10 times greater than that of all other native benthic invertebrates. When zebra mussels invade, benthic invertebrate communities change dramatically in terms of total biomass, species composition, and relative abundance of functional groups. Native filter feeders are outcompeted by *D. polymorpha* and decrease in abundance, while animals feeding on the sediments increase in abundance. Although *D. polymorpha* can cause a dramatic decline in the abundance of unionids, after initial peaks in zebra mussel abundance, *D. polymorpha* coexist with unionids. *Dreissena* are very effective filter feeders and shift materials from the pelagic to the benthos through their filter feeding and deposition of pseudofeces. When zebra mussels invade phytoplankton and zooplankton abundance decreases, the biomass of benthophage fish increases, and a greater percentage of the primary productivity is consumed by higher trophic levels than in systems without zebra mussels.

KEY WORDS: Zebra mussels, freshwater ecosystems, benthic-pelagic coupling, benthic communities, unionids

INTRODUCTION

The zebra mussel, *Dreissena polymorpha* Pallas (1771), was found across Europe before the last glaciation (Starobogatov and Andreeva 1994). The Caspian Sea, the Black Sea Basin, the Azov Sea, and lower parts of rivers feeding them formed the postglacial distribution of the zebra mussel until early in the 19th century, when shipping canals for transportation and commerce were developed from the Black Sea basin to the Baltic Sea basin (Köppen 1883, Andrusov 1897, Arwidsson 1926, Ovchinnikov 1933, Deksbakh 1935, Zhadin 1946, Mordukhai-Boltovskoi 1960). The zebra mussel invaded through new waterways, primarily through the Dnieprovsko-Nemansky route, which connected the Dnieper (Black Sea basin) and the Neman rivers (Baltic Sea basin) (Starobogatov and Andreeva 1994), and the canal that connected the Dnieper and Zapadnyi Bug rivers (Kinzelbach 1992). The first ships traveled the Dnieper-Neman Canal in 1804 and Dnieper-Zapadnyi Bug Canal in 1775. By 1824, zebra mussels were found in England, and by 1825, they were found in eastern Prussia (Starobogatov and Andreeva 1994). Zebra mussels continued to spread rapidly through the freshwaters of Eurasia. Today, new lakes and rivers in eastern and western Europe are still being invaded (Gérondet 1966, Lyakhnovich et al. 1984, Karatayev 1989, Kinzelbach 1992, and others). Although *Dreissena bugensis* (quagga mussel) has invaded the Ukraine and spread in the South Bug and Dnieper River basins (Starobogatov and Andreeva 1994), only *D. polymor-*

pha has invaded northwestern former Soviet Union (FSU), including Belarus, and has been the major target of study by FSU scientists.

There is a long, rich history of research on *Dreissena* in the FSU and eastern Europe, focusing on taxonomy, biology, food web ecology, productivity, and ecosystem function. Problems associated with electric power plants, industry, and municipal water supplies due to invasions of *Dreissena* after World War II stimulated research on the biology and control of this invading species. In addition, in the 1970s, zebra mussels and several other freshwater taxa were targeted for study under a project that was part of an international research program coordinated by UNESCO, the Man and Biosphere Program (Starobogatov 1994). This project stimulated further studies of the ecological roles and effects of *D. polymorpha*. Given this extensive history of research, we have long-term data on both pre- and post-zebra mussel invasion communities for a variety of waterbodies. In addition, many studies have been conducted on specific lakes at different stages of invasion, early when zebra mussel populations and biomass are highest, and later when they decline. This has allowed us to determine the role and function of *Dreissena* during all phases of invasion and in different types of waterbodies.

Unfortunately, because of language and political barriers, this extensive body of work has not been readily available to North America and English-speaking scientists. Our goal is to provide access to this information. We summarize 60 y of research conducted to elucidate the role and function of *D. polymorpha* in freshwater systems studied in the FSU and eastern Europe. We

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consider how zebra mussels alter benthic communities both by their feeding activities and by creating a new habitat type for benthic species, and how their filtering affects planktonic species and food web interactions. We address the ecological role of zebra mussels as filter feeders. In many cases, differences in methodologies have made direct comparisons of studies difficult. We address this issue by converting existing data to similar units (where possible) for direct comparison and make recommendations for preferred methods and units for future research.

EFFECT ON BENTHIC COMMUNITIES

In benthic communities within their native distribution, such as the brackish waters of the Caspian Sea, Aral Sea, and Azov Sea, *Dreissena* generally are not the dominant species. For example, in Taganrog Bay of the Azov Sea, the bivalve *Monodacna colorata* is the dominant species and is only occasionally codominant with *D. polymorpha* (Vorobiev 1949, Nekrasova 1971). In the northern part of the Caspian Sea, *D. polymorpha* and *Dreissena rostriformis* comprise only 25% of the bivalve biomass; *Didacna* and *Monodacna* are typically more abundant (Shorygin and Karpevich 1948). In freshwaters, where they are the only bivalves that attach to hard substrates and have a planktonic larval stage, dreissenids, especially *D. polymorpha*, can become enormously abundant and, within a short period of time, can obtain a biomass 10 times greater than that of all other native benthic invertebrates (Sokolova et al. 1980a, Shevtsova and Kharchenko 1981, Karatayev 1983, Kharchenko 1983, Karatayev 1988, Karatayev and Lyakhovich 1988, Lyakhovich et al. 1988, Kharchenko 1990, Protasov and Afanasiev 1990, Karatayev 1992, Karatayev et al. 1994a, Sinitsyna and Protasov 1994, Karatayev and Burlakova 1995a). When outside its original distribution, the zebra mussel is frequently competitively dominant over native freshwater fauna and has large effects on all parts of the ecosystem, especially benthic animals (Dusoge 1966, Wiktor 1969, Wolnomiejski 1970, Sokolova et al. 1980b, Kharchenko and Protasov 1981, Karatayev 1983, Karatayev et al. 1983, Afanasiev 1987, Karatayev and Lyakhovich 1988, Karatayev 1992, Karatayev et al. 1994a, Karatayev and Burlakova 1995a).

Changes in Benthic Community With Zebra Mussels

By aggregating in large densities, *D. polymorpha* create new two- and three-dimensional habitats for different organisms, and pseudofeces and feces provide an abundant food supply for detritivores (Izvekova and Lvova-Kachanova 1972, Lvova-Kachanova and Izvekova 1973, Spiridonov 1976, Lvova et al. 1980, Sokolova et al. 1980b, Karatayev 1983, Karatayev et al. 1983, Kharchenko 1983, Karatayev 1988, Karatayev and Lyakhovich 1988, Kharchenko 1990, Karatayev et al. 1994a, Slepnev et al. 1994). In addition, water flow induced by zebra mussel filtering improves oxygen conditions in the benthos. As a consequence, a different community forms in mussel aggregations.

The complex of *Dreissena* and its associated species forms a coherent, biologically generated interactive community, which has been called a consortium (Kharchenko and Protasov 1981, Kharchenko 1990, Karatayev et al. 1994a). Although a variety of animals can have a similar function in marine systems (e.g., marine mussel beds, coral reefs), only *Dreissena* has this role in freshwater. According to Kharchenko and Protasov (1981), there are several direct functional relationships between *Dreissena* and associated species: (1) formation of habitat (*Dreissena* create a habitat for

benthic species); (2) trophic relationships (*Dreissena* and their associates can have mutually beneficial feeding associations); (3) material relationships (*Dreissena* provide the materials, such as shell fragments, byssus, and small mussels, used by associated species for the construction of houses); (4) dispersal relationships (*Dreissena* can be transported by associated taxa).

The relationship between *D. polymorpha* and infaunal taxa may not be simple. Some species may be positively affected by *D. polymorpha*, while others are negatively affected (Sebestyen 1937, Dusoge 1966, Wolnomiejski 1970, Sokolova et al. 1980b, Karatayev 1983, Karatayev et al. 1983, Afanasiev 1987, Karatayev and Lyakhovich 1990, Karatayev and Burlakova 1992, Karatayev et al. 1994a, Slepnev et al. 1994). We use studies done in Lukomskoe lake (Belarus), where isolated aggregations of zebra mussels, or druses, form in the sandy littoral zone, to demonstrate the effects of *D. polymorpha* on benthic communities (Karatayev 1983, Karatayev et al. 1983, Karatayev 1988, Karatayev et al. 1994a). Similar patterns have been observed in most other waterbodies studied (Dusoge 1966, Wiktor 1969, Wolnomiejski 1970, Kharchenko and Protasov 1981, Afanasiev 1987, Karatayev et al. 1994a, Slepnev et al. 1994, and others).

To assess the effect of druses on the benthic community, Karatayev and his associates sampled both sand and druse communities. The presence of individual *D. polymorpha* on the bottom did not change the qualitative and quantitative composition of the benthic community (Karatayev 1983, Karatayev et al. 1983, Karatayev 1988, Karatayev et al. 1994a). However, in the presence of druses, the community changed radically. Species density and community composition depended on the size of druses. With increasing druse size, species richness increased and then stabilized in druses of more than 80 individual mussels. Forty-eight species and higher taxa of benthic animals were identified in the absence and presence of druses, but only 26 appeared to be members of both communities. In the sandy community, chironomids and oligochaetes were the most common taxa, as in preinvasion communities. The majority were small animals that live within the sediment. In the zebra mussel aggregations, the benthic community was composed of larger animals such as snails, amphipods, isopods, trichoptera, and leeches. For each species in each habitat type, they calculated a dominance index, $P \cdot \sqrt{B}$ (Mordukhai-Boltovskoi 1940) where P is the percentage of samples with a given species, and B is the average biomass of that species across all samples. The dominant species in the sand habitat was the chironomid *Stictochironomus psammophilus*, whereas in the druses, only a single individual of this species was found. The snail *Limnaea lagotis* and the amphipod *Gammarus lacustris* were dominant in zebra mussel druses, whereas in the sand community, only a single *L. lagotis* and no *G. lacustris* were found. Subdominant taxa were also dissimilar between these two habitat types (Karatayev 1983, Karatayev et al. 1983, Karatayev 1988, Karatayev et al. 1994a).

Total benthic density in sandy sediments was $40,995 \pm 3,263 \text{ m}^{-2}$, and total wet biomass was $15.1 \pm 1.0 \text{ g m}^{-2}$. In *D. polymorpha* druses (without including mussels), the density of benthic animals was $27,536 \pm 4,085 \text{ m}^{-2}$, and the biomass was $114.8 \pm 20.0 \text{ g m}^{-2}$. Invertebrate biomass was 8 times greater in druses, even though densities were 1.5 times lower than in sandy sediments because the community consisted of larger species. Therefore, a new community, not generally found in sandy sediments, forms in *D. polymorpha* druses, and the typical sandy sediment

community disappears, creating a mosaic pattern in the benthos (Karatayev 1983, Karatayev et al. 1983, Karatayev 1988, Karatayev et al. 1994a).

Slepnev et al. (1994) compared invertebrate colonization of zebra mussel druses and rubber models of druses in a cooling pond for the Krivoy Rog Power Plant (Ukraine). They found that after 14 days of exposure, the total density of invertebrates in the containers with *D. polymorpha* was significantly higher ($346,687 \pm 56,276 \text{ m}^{-2}$) than that in containers containing rubber models ($116,000 \pm 20,335 \text{ m}^{-2}$). The largest differences were found for Cyclopidae (19.7 times greater), *D. polymorpha* veligers (9.9 times greater), and Chydoridae (5 times greater) (Slepnev et al. 1994).

Kharченко and Protasov (1981), using the Shannon index, found that the diversity of benthic communities increased more than two times in the presence of *Dreissena* (*D. polymorpha* + *D. bugensis*) in the North-Crimean Canal (Ukraine). They also found substantial increases in species richness, density, and biomass of benthic fauna. Shevtsova and Grigorovich (1989) found the same result in the Dnieper-Donbass Canal (Ukraine).

Karatayev and Lyakhovich (1990) found that without *D. polymorpha*, the crustaceans *Asellus aquaticus* and *G. lacustris* populated the shallow littoral zone of Lukomskoe lake. At depths over 2 m, these two species were only found with *D. polymorpha*, and the density of *A. aquaticus* was positively correlated with the density of *D. polymorpha* ($r = 0.7$) (Karatayev and Lyakhovich 1990).

Similar patterns have been found in other European studies. Dusoge (1966) found that the abundance of benthic invertebrates in Mikolajskie Lake (Poland) was positively correlated with zebra mussel abundance. Afanasiev (1987) found positive correlations between zebra mussel biomass and the density of some oligochaetes in the cooling reservoir of a power plant (Ukraine) (e.g., *Aulodrilus limnobiis*, $r = 0.98$; *Psammoryctides albicola*, $r = 0.99$; *Limnodrilus hoffmeisteri*, $r = 0.98$), but for others, he found no correlation or negative correlation (e.g., *Nais bretscheri*; $r = -0.76$). Wolnomiejski (1970) reported that *D. polymorpha* provide substrate or shelter for many benthic taxa, including the isopod, *A. aquaticus*, larval chironomids *Microtendipes* gr. *chloris* and *Limnochironomus* gr. *nervosus*, and the leech *Helobdella stagnalis*. Also, Wiktor (1969) found that the benthic biomass near colonies of *D. polymorpha* was twice that found elsewhere. Spatially complex groups of *D. polymorpha* create favorable microhabitats for small organisms as well. The abundance of oligochaetes in the Chernobyl Nuclear Power Station cooling pond (Ukraine) increased from 17,000 to 39,000 m^{-2} with the addition of *D. polymorpha*, and densities were significantly correlated with *D. polymorpha* biomass (A. A. Protasov and O. O. Sinitsyna, personal communication, Institute of Hydrobiology Ukrainian Academy of Sciences, Kiev, Ukraine).

In North America, zebra mussels seem to have similar effects on benthic communities (Stewart and Haynes 1994, Wisenden and Bailey 1995, Botts et al. 1996). Wisenden and Bailey (1995) found that the density of macroinvertebrates in Lake Erie associated with higher densities of *D. polymorpha* (720.8 m^{-2}) was $3,452.2 \pm 1,192.3 \text{ m}^{-2}$, and the taxonomic richness was 6.0 ± 0.6 ; the density of invertebrates associated with low densities of *D. polymorpha* (152.2 m^{-2}) was $411.6 \pm 58.7 \text{ m}^{-2}$, and the taxonomic richness was 3.1 ± 0.5 . Botts et al. (1996) compared the density of benthic invertebrates in bare sand and in sand associated with zebra mussel druses in Lake Erie. In a survey, they found that the densities of amphipods, chironomids, oligochaetes, turbellarians, hydrozoans,

and the total invertebrate density were significantly higher in sand with druses than in bare sand. In an experiment with mesh bags containing either living druses, artificial druses made from cleaned zebra mussels shells, or no zebra mussels, they found that chironomids were significantly more abundant in living druses than in nonliving druses. There were no significant differences in oligochaetes among all three treatments. Therefore, it seems that North American and FSU benthic invertebrate communities respond similarly to the addition of zebra mussels.

Trophic Shifts

When zebra mussels invade, the development of a large population of effective filter feeders causes a radical shift in the benthic trophic structure (Lvova-Kachanova and Izvekova 1978, Sokolova et al. 1980a, Sokolova et al. 1980b, Karatayev 1992, Karatayev and Burlakova 1992, Karatayev et al. 1994a). Native filter feeders are outcompeted by *D. polymorpha* and decrease in abundance, whereas animals feeding on the sediments increase in abundance (Karatayev and Burlakova 1992, Karatayev et al. 1994a). Studies of Lukomskoe lake provide the most complete information on the affect of *D. polymorpha* on the trophic structure of invertebrate communities (Karatayev and Burlakova 1992). The feeding mode of 117 of the 245 species living in Lukomskoe lake were determined from the literature (Burlakova, unpublished data). These 117 species constitute more than 99% of the total biomass of benthic invertebrates in this lake. Karatayev and Burlakova (1992), using data from Lyakhovich et al. (1982), collected in 1968 and 1969 for information on the community before the zebra mussel invasion, and Karatayev (1983) data from 1978 (8 y after *D. polymorpha* invaded), determined the trophic structure of the zoobenthos before and after zebra mussel invasion. Using a classification scheme based on feeding characteristics developed by Izvekova (1975), the community of benthic invertebrates was divided into the following trophic groups: I, detritus filterers; II, detritus filterers + gatherers; III, detritus gatherers; IV, deposit feeders; V, omnivorous gatherers + grabbers; VI, predators-active grabbers. This scheme is similar to that developed by Cummins (1978), but differs in some important ways. The first four groups in Izvekova's scheme (detritus filterers, detritus filterers + gatherers, detritus gatherers, and deposit feeders) are equal to Collectors in Cummins' scheme. The fifth group in Izvekova's classification (omnivorous gatherers + grabbers) includes Shredders and Scrapers in Cummins' classification. The sixth group (predators-active grabbers) is equal to Piercers and Engulfers (predators) in Cummins' classification.

Before the appearance of *D. polymorpha*, the littoral zone was dominated by detritus gatherers (III) and detritus filterers (I), mainly snails and bivalves (Table 1). Other trophic groups were not a significant part of the community. The trophic structure of the profundal zone was more complex and included predators-active grabbers (VI, larval chironomids, *Procladius choreus*, and *Chaoborus*), deposit feeders (IV), and detritus filterers + gatherers (II, mainly *Chironomus plumosus*). For the whole lake, the most important groups were the detritus gatherers (III) and detritus filterers (I) (Karatayev and Burlakova 1992).

After the invasion of *D. polymorpha*, without including *D. polymorpha*, the role of detritus gatherers (III) in the littoral zone increased because of the expansion of their food supply, organic matter deposited by *D. polymorpha*. The number of predators-active grabbers (VI) and omnivorous gatherers + grabbers (V) also increased. The proportion of native detritus filterers (I) decreased

TABLE 1.

Trophic structure of the zoobenthos of Lukomskoe lake before and after the appearance of zebra mussels.

Trophic Group		Littoral Zone			Profundal Zone			Whole Lake		
		Preiovasion	Postinvasion		Preinvasion	Postinvasion		Preinvasion	Postinvasion	
			Without Zebra Mussels	With Zebra Mussels		Without Zebra Mussels	With Zebra Mussels		Without Zebra Mussels	With Zebra Mussels
I	Detritus filterers	43.7	5.1	95.9	8.1	5.5	7.5	40.9	5.3	94.8
II	Detritus filterers + gatherers	0.3	1.4	0.1	21.7	7.5	7.4	2.0	3.0	0.2
III	Detritus gatherers	48.2	71.3	3.1	8.3	59.6	58.3	45.2	69.7	3.8
VI	Deposit feeders	6.8	8.3	0.3	21.8	18.4	18.0	7.9	10.9	0.6
V	Omnivorous gatherers + grabbers	0.5	3.6	0.2	0.3	2.8	2.7	0.4	3.2	0.2
VI	Predators-active grabbers	0.5	10.3	0.4	39.8	6.2	6.1	3.6	7.9	0.4

Cell values are the percentage of the total benthic biomass (g) found in each trophic group (from Karatayev and Burlakova 1992).

eightfold. In the profundal zone, the dominant group of benthic animals was detritus gatherers (III) and then deposit feeders (IV). The biomass of detritus filterers + gatherers (II), detritus filterers (I), and predators-active grabbers (VI) decreased relative to pre-invasion communities. Except for *D. polymorpha*, the community was dominated by animals using food from sediments (Karatayev and Burlakova 1992, Karatayev et al. 1994a).

If *D. polymorpha* is considered with the rest of the benthic community, the trophic structure of the benthic community is characterized by an extremely high dominance of one trophic group—detritus filterers (I)—which accounts for 95% of the total biomass of benthic invertebrates (Karatayev and Burlakova 1992). As a result, the trophic structure of the littoral zone is reduced, and the remaining trophic groups contribute relatively small amounts of biomass to the total. The filter feeding species, which were dominant before the zebra mussel invasion, disappeared or became rare and gave way to detritus gatherers (III), predators-active grabbers (VI), and deposit feeders (IV) (Karatayev and Burlakova 1992, Karatayev et al. 1994a).

Similar patterns have been observed in four other waterbodies across the FSU (Karatayev et al. 1994a) (Table 2). Similar changes in trophic structure were found in Uchinskoe reservoir, where the invasion of *D. polymorpha* resulted in drastic changes in the benthos (Lvova-Kachanova and Izvekova 1978, Sokolova et al. 1980a, Sokolova et al. 1980b). *D. polymorpha* replaced the dominant species (the chironomid *Glyptotendipes paripes*) and changed the relative abundance of different species and trophic groups, especially chironomids that collect loose detrital particles (gatherers), who became most abundant in zebra mussel commu-

nities. Sokolova et al. (1980b) hypothesized that, as a result of the constant water exchange created by *D. polymorpha*, a habitat similar to nearshore benthos is created in druses. This would allow littoral zone species, such as the chironomid *Microtendipes pedilus*, which move from nearshore when water levels fluctuate to inhabit zebra mussel-dominated areas. Thus, the shift in benthic trophic structure induced by *D. polymorpha* in Lukomskoe lake is typical of that in waterbodies with established zebra mussel populations across the FSU (Karatayev et al. 1994a).

Geographic Clines in Benthic Communities With Zebra Mussels

Because zebra mussels occur over such vast geographic areas, the species they are associated with change with geographic region. To determine if the role of *D. polymorpha* is the same in spite of large faunal differences associated with different geographic areas, Karatayev et al. (1994a) analyzed data collected for six waterbodies located in different climatic zones of FSU: Lukomskoe lake (55°N), Volgogradskoe (49°N) and Tsimlyanskoe (48°N) reservoirs, Dniester (46°N) and Dnieper Bug (47°N) Limans (coastal brackish lakes), and Taganrog Bay (47°N) of the Sea of Azov (Karatayev et al. 1994a). Over 100 species and higher taxa of macroinvertebrates are found in all of these zebra mussel communities, and 46 occur more than once; thus, when *D. polymorpha* spread north, beyond the limits of their original distribution, they also spread beyond the distribution of their natural complex of associated species (i.e., inhabitants of Ponto-Caspian basin).

The most diverse benthic taxa found in zebra mussel commu-

TABLE 2.

Trophic structure of zoobenthos of zebra mussel communities from different waterbodies across the FSU.

Trophic Group		Lukomskoe Lake	Tsimlyanskoe Reservoir	Dniester Liman	Dnieper-Bug Liman	Taganrog Bay
I	Detritus filterers	94.8	93.1	93.3	97.1	93.7
II	Detritus filterers + gatherers	0.2	3.7	3.6	1.6	4.0
III	Detritus gatherers	3.8	0.1	0.1	0.3	0.0
IV	Deposit feeders	0.6	1.1	2.8	0.6	0.3
V	Omnivorous gatherers + grabbers	0.2	1.9	0.1	0.3	0.3
VI	Predators-active grabbers	0.4	0.1	0.1	0.2	0.1

Cell values are the percentage of the total benthic biomass (g) found in each trophic group (from Karatayev et al. 1994a).

nities were: Crustacea (22 species), Chironomidae (8 species), Gastropoda (4 species), and Oligochaeta (4 species) (Karatajev et al. 1994a). Crustaceans play particularly important roles in zebra mussel communities across Europe. Cumaceans (*Pterocuma pectinata*, *Schizorhynchus endorelloides*) dominate in waterbodies close to the original geographic distribution of zebra mussels. Further north, amphipods, especially gammarids, are dominant (Karatajev et al. 1994a). High densities of gammarids have also been found associated with *D. polymorpha* in other European waterbodies (Kharchenko 1983, Shevtsova and Grigorovich 1989, Protasov and Afanasiev 1990) and in North America (Stewart and Haynes 1994, Wisenden and Bailey 1995, Botts et al. 1996).

Although the total species composition varied, *D. polymorpha* had an extremely high dominance index ($P \cdot \sqrt{B}$) in all communities studied. As a rule, when *D. polymorpha* are in freshwater, they are the single, dominant benthic species in terms of biomass, with a biomass 10–50 times greater than the total mass of all other benthic invertebrates in these communities (Sokolova et al. 1980a, Shevtsova and Kharchenko 1981, Karatajev 1983, Kharchenko 1983, Karatajev 1988, Karatajev and Lyakhnovich 1988, Lyakhnovich et al. 1988, Kharchenko 1990, Protasov and Afanasiev 1990, Karatajev 1992, Karatajev et al. 1994a, Sinitsyna and Protasov 1994, Karatajev and Burlakova 1995a).

Affects on Unionids

Before the invasion of *D. polymorpha*, the only large bivalves in freshwater benthic communities were unionids (superfamily Unionacea). Unionids have a very different lifestyle and life history than *D. polymorpha*. They live in soft sediment, crawl through sediment with a large foot, live solitary or in groups (but not in as extreme densities as *D. polymorpha*), have slow growth, have low fecundity, are long lived, and have parasitic glochidia larvae (McMahon 1991). Unionids can provide the most abundant source of hard substratum for the colonization of *D. polymorpha* in many lakes, reservoirs, and rivers (Sebestyen 1937, Zhadin and Gerd 1961, Wiktor 1963, Biryukov et al. 1964, Kuchina 1964, Wolff 1969, Lewandowski 1976, Karatajev 1983, Karatajev and Tishchikov 1983, Arter 1989, Karatajev and Burlakova 1995b). By attaching to their valves, *D. polymorpha* can make it more difficult for unionids to burrow and move through sediment, and the added mass of *D. polymorpha* can weigh down unionids, resulting in burial in very soft or unconsolidated sediments (Karatajev 1983, Karatajev and Tishchikov 1983). Mussel attachment to unionid valves can increase drag and the likelihood of dislodgment by water motion for species living nearshore (Karatajev 1983, Karatajev and Tishchikov 1983). In addition, zebra mussel attachment can occlude the openings in unionid valves, either preventing opening, for filtration and feeding, or closing the valves. *D. polymorpha* directly compete with unionids for food and occupy otherwise available space.

Many European scientists have found that *D. polymorpha* attached to living unionids more frequently than to other substrates (Sebestyen 1937, Biryukov et al. 1964, Wolff 1969, Lewandowski 1976, Karatajev 1983, Karatajev and Tishchikov 1983, Karatajev and Burlakova 1995b). Similar patterns have been found in North America in Lake St. Clair (Hebert et al. 1989).

The density of *D. polymorpha* in druses attached to living unionids is much higher than in those found on any other substrates within the same region of the littoral zone, including stones and empty valves (Karatajev 1983, Karatajev and Tishchikov

1983). This may indicate that live unionids provide better living conditions for *D. polymorpha*. Lewandowski (1976) found a strong correlation between the degree of overgrowth of unionids by zebra mussels and the average density of zebra mussels in lakes in Poland. In addition, he found that the mass of shells of *Anodonta piscinalis* heavily overgrown by *D. polymorpha* was significantly higher than the mass of shells of similar-sized unionids without *D. polymorpha*.

Unionids can actively move to areas with good food and oxygen conditions and, by mixing the water while filtering, can improve the local food and oxygen conditions for attached *D. polymorpha* (Karatajev 1983, Karatajev and Tishchikov 1983). However, overgrowth by zebra mussels adversely affects the host unionid. The extent of this effect depends on a number of factors, including: (1) time since invasion of *D. polymorpha* (Sebestyen 1937, Dussart 1966, Karatajev and Burlakova 1995b); (2) type of bottom sediment (Arter 1989, Karatajev and Burlakova, unpublished data); (3) unionid species (Lewandowski 1976, Arter 1989, Haag et al. 1993, Strayer and Smith 1996); and (4) unionid sex (Haag et al. 1993).

Extensive overgrowth by *D. polymorpha* of unionids, resulting in mass mortality, is characteristic of periods of rapid population growth of zebra mussels when they invade a new waterbody (Sebestyen 1937, Dussart 1966, Karatajev and Burlakova 1995b). Subsequent to this period, *D. polymorpha* coexist with native bivalves in FSU freshwaters. Although overgrowth can cause some host mortality, populations of unionids are not only preserved, but also can maintain high densities (Karatajev 1983, Karatajev and Tishchikov 1983, Miroshnichenko et al. 1984, Miroshnichenko 1987).

In the profundal zone of Lukomskoe lake, dominated by silt, *D. polymorpha* were found only on unionids. On average, about 20% of the total density and biomass of *D. polymorpha* in this lake were attached to living unionids (Karatajev 1983). Lewandowski (1976) found similar patterns in Mikolajskie Lake (Poland): *D. polymorpha* inhabited 85% of the unionids, and the total mass of attached *D. polymorpha* exceeded the mass of host unionids 35% of the time. Although there was a drop in the number of unionid species in Mikolajskie Lake from 1972 to 1987 from 5 (*Unio tumidus*, *Unio pictorum*, *Anodonta piscinalis*, *Anodonta cygnea*, and *Anodonta complanata*) to 3 (*U. tumidus*, *U. pictorum*, and *A. piscinalis*), this drop was not a direct result of zebra mussel effect (Lewandowski 1991). During this same time, the average density of zebra mussels declined from more than 2,000 m⁻² to less than 100 m⁻² (Stanczykowska and Lewandowski 1993). The decline in unionid diversity and zebra mussel density was attributed to increasing lake eutrophication and pollution (Lewandowski 1991).

In the Tsimlyanskoe reservoir (Russia), unionids (mainly *U. pictorum* and *A. cygnea*) successfully coexist with *D. polymorpha* (Miroshnichenko et al. 1984, Miroshnichenko 1987). Since 1960, the average annual biomass over the entire reservoir was 571 g m⁻² for *D. polymorpha*, 88 g m⁻² for *U. pictorum*, and 46 g m⁻² for *A. cygnea* (Miroshnichenko et al. 1984). According to Ponyi (1992), in Lake Balaton, the average density of unionids before the zebra mussel invasion (1932) was 3 m⁻²; between 1966 and 1968, it was 2 m⁻². The decline in the abundance of unionids (*U. tumidus*, *U. pictorum*, *A. cygnea*) in Lake Hallwil (Switzerland) from the 1910s to the 1980s was explained by a decrease in the number of host fish for unionid larvae, an increase in eutrophication, and the influence of *D. polymorpha*, which colonized the lake in the 1970s (Arter 1989).

The effect of *D. polymorpha* on unionids may depend on the

type of bottom sediment. In the Svisloch river (Belarus), sandy and rubble sediments alternate with silt. In sandy and rubble areas, unionids have up to 100 attached *D. polymorpha* per individual, whereas in silt, unionids bury in sediments and are completely free of zebra mussels, even though the density of unionids was up to 100 m⁻² (Karatayev and Burlakova, unpublished data). Arter (1989) found that in Lake Hallwil, *U. tumidus* is usually buried in the sediments and is rarely overgrown by zebra mussels. However, *A. cygnea* is often only partly buried and is colonized more often by zebra mussels.

Although the appearance of *Dreissena* in the North American waterbodies has been correlated with negative effects on aboriginal unionids (Haag et al. 1993, Gillis and Mackie 1994, Nalepa 1994, Schloesser and Nalepa 1994), a large decline in the diversity and abundance of unionids was detected long before the appearance of *Dreissena* (Nalepa et al. 1991, Schloesser and Nalepa 1994). Will *Dreissena* have greater effects on unionids in North America than in Europe? In preglaciation Europe, *Dreissena* and

unionids coexisted. Because North American aboriginal species have no evolutionary history of coexistence with *Dreissena*, *Dreissena* may have a larger effect on North American than European species. The species composition of unionids in North America is much different than that in Europe, and there may be species-specific differences in response to fouling. The bivalve fauna of North American freshwaters is the most diverse in the world, consisting of 250 native and 6 introduced species, 227 of which belong to the superfamily Unionacea (families Margaritiferidae and Unionidae) (McMahon 1991). The bivalve fauna in Europe consists of 62 species and only 14 species in the superfamily Unionacea (Jaekel 1967).

North American scientists have reported extremely high densities of *D. polymorpha*, more than several thousand per unionid (Hebert et al. 1991, Schloesser and Kovalak 1991, Gillis and Mackie 1994, Schloesser and Nalepa 1994). These densities are much higher than those reported by European scientists (Table 3). Do these differences constitute a significant difference in the

TABLE 3.
Effect of zebra mussels on unionids.

Location	% of Unionids Colonized	Number of Zebra Mussels per Host Unionid	Biomass of Zebra Mussels per Host Unionid (g)	Ratio of Mass of Zebra Mussels and Host Unionid	References
Europe: rapid growth of zebra mussel population					
Naroch lake, 1990	60	9.5	1.8	0.3	Karatayev and Burlakova 1995b
		(1-90)	(0.3-12.4)	(0.04-1.7)	
Naroch lake, 1993	100	135	34.0	2.8	Karatayev and Burlakova, unpublished data
		(41-200)	(9.5-46.1)	(0.3-6.6)	
Drozdy reservoir, 1995	100	38.1	12.6	1.8	Karatayev and Burlakova, unpublished data
		(7-280)	(3.9-57.4)	(0.5-11.0)	
Europe: established zebra mussel population					
Lukomskoe lake, 1978	75	40	7.6	1.2	Karatayev and Tishchikov 1983
		(2-216)	(0.2-49.8)	(0.04-9.10)	
Myastro lake, 1993	94	10	5.9	0.6	Karatayev and Burlakova, unpublished data
		(1-23)	(1.3-11.8)	(0.2-1.2)	
Mikolajskie Lake, 1972	85	20			Lewandowski 1976
		(<99)			
Mikolajskie Lake, 1974	92	52			Lewandowski 1976
		(<132)			
North America					
Lake St. Clair, 1989		5,496		2-3	Hebert et al. 1991
(1 site only)		(<10,520)			
Lake St. Clair, 1990	97			1.2	Nalepa 1994
(avg. of 15 sites)		(0-1,360)		(0-8.5)	
Western Lake Erie, September 1989	100	6,805	36	2/6	Schloesser and Nalepa 1994
		(<11,550)			
Western Lake Erie, May 1990	100	346	9	0.5	Schloesser and Nalepa 1994
Lake Erie, Power Plant Canal, August 1989	100	6,777	44.8	0.74	Schloesser and Kovalak 1991
		(2,491-10,732)	(30.0-54.9)	(0.46-3.79)	
Lake Clark, Michigan May 1996	100	219	5.8	0.40	Karatayev and Burlakova, unpublished data
		(60-473)	(1.9-13.6)	(0.08-0.97)	
Lake Vineyard, Michigan May 1996	100	118	16.1	0.22	Karatayev and Burlakova, unpublished data
		(34-179)	(3.8-34.9)	(0.07-0.41)	

Cell values are means. Ranges are in parentheses.

effect of zebra mussels on native unionids? From the perspective of the unionid, the mass of attached *D. polymorpha*, or the ratio of the mass of attached zebra mussels to the mass of the host unionid, is probably more important than density. Unfortunately, North American scientists rarely report their data in terms of mass. Of the studies that we could compare with European data, the mean ratio between the biomass of attached *D. polymorpha* and the host unionid was very similar to that found in Europe (Table 3).

Differences between the density of attached mussels reported by North American and European scientists could result if the size-frequency composition of zebra mussel populations is much smaller in North America, or if North American scientists include smaller mussels in their estimates of density than European scientists. In general, European scientists do not include mussels smaller than 1 or 2 mm in density estimates (Lvova 1977, Lvova 1980, Karatayev 1983, Lyashenko and Kharchenko 1988, Lvova et al. 1994, and others); however, sometimes they do not include mussels smaller than 5 (Bij de Vaate 1991) or 8 mm (Hamburger et al. 1990). The overwinter mortality of young-of-the-year and 1-y-old mussels is very high, and by the spring, the number of live mussels is greatly reduced. For example, in western Lake Erie in February 1989, the density of *D. polymorpha* was 24 ± 3.9 per unionid, and in August, after larval settlement, the density of mussels averaged 6.777 ± 811 per unionid (Schloesser and Kovalak 1991). In September 1989, the mass of mussels attached to unionids was three times greater than the unionid mass. By May and June 1990, the mass of attached mussels dropped to one-third of host unionid mass (Schloesser and Nalepa 1994).

Currently, North America is in the early phase of zebra mussel invasion, and populations are growing rapidly. At this stage of invasion, *D. polymorpha* caused a dramatic decline in the abundance of unionids in Europe (Sebestyen 1937, Dussart 1966, Karatayev and Burlakova 1995b). However, to our knowledge, the zebra mussel invasion did not result in the complete disappearance of unionids in any European lakes. After initial peaks in zebra mussel abundance, *D. polymorpha* coexist with unionids in all lakes, reservoirs, and rivers studied. Will this pattern hold true for

North America? With time, perhaps the effect of *D. polymorpha* on unionids will decrease.

EFFECT ON PELAGIC COMMUNITIES

Filtering Rate

Dreissena are filter feeders, capable of filtering large quantities of water in a relatively short period of time. Many of the effects of zebra mussels on freshwater ecosystems are linked to their filtering. They circulate water for respiration and feeding and remove particles from the water, which are either consumed or bound as pseudofeces and expelled to the benthos. Although many researchers have investigated the filtering of *D. polymorpha* (Voskresenski 1957, Kondratiev 1962, Mikheev 1966, Mikheev 1967a, Mikheev 1967b, Stanczykowska 1968, Kondratiev 1969, Kondratiev 1970; Morton 1971, Lvova 1977, Reeders and Bij de Vaate 1990, Karatayev and Burlakova 1993, Karatayev and Burlakova 1994), standardized methodology has not been used, and often, experimental setups are not adequately described to permit direct comparisons of results.

Filtering rates of *Dreissena* can be difficult to measure in the laboratory, and therefore, experimental design can affect results. If filtration cannot be measured in a flow-through system where concentrations of particles are held constant, it is recommended that experiments be in relatively large volumes of water and be of short duration such that the concentration of particles is not depressed more than 20–30% during the entire experiment (Zihon-Lukanina et al. 1990). In addition, experimenters must consider the differential filtration of particles of different sizes and qualities. Filtration estimates for natural seston may be much different than for single-species cultures or inert particles (Table 4).

FSU scientists generally calculate the filtering rate of *D. polymorpha* based on shell length or wet total mass (WTM, shell plus soft tissue) (Kondratiev 1962, Mikheev 1966, Mikheev 1967a, Mikheev and Sorokin 1966, Kondratiev 1969, Lvova 1977, Karatayev and Burlakova 1993, Karatayev and Burlakova 1994, Karatayev and Burlakova 1995b), as do many other Europeans

TABLE 4.
Filtering rate of zebra mussels from various studies.

Reference	Food	Temperature (°C)	Filtering Rate (mL g WTM ⁻¹ h ⁻¹)	Author's Units
Europe				
Kondratiev 1962	Natural seston	16–17	43	mL g WTM ⁻¹ h ⁻¹
Mikheev and Sorokin 1966	<i>Chlorella</i>	n.r.	69	mL ind. ⁻¹ h ⁻¹
Stanczykowska 1968	Natural seston	17–20	35	mL g WTM ⁻¹ h ⁻¹
Lvova 1977	Natural seston	17–20	40	mL g WTM ⁻¹ h ⁻¹
Dorgelo and Smeenk 1988	<i>Chlamydomonas eugametos</i>	15	35	mL ind. ⁻¹ h ⁻¹
Reeders and Bij de Vaate 1990	Natural seston	10–21	83	mL ind. ⁻¹ h ⁻¹
Wisniewski 1990	Natural seston	n.r.	110	mL ind. ⁻¹ h ⁻¹
Karatayev and Burlakova 1993	Natural seston	20	66	mL g WTM ⁻¹ h ⁻¹
Karatayev and Burlakova 1995a	Natural seston	20	38	mL g WTM ⁻¹ h ⁻¹
North America				
Bunt et al. 1993	<i>Cryptomonas</i> sp.	20	49	mL ind. ⁻¹ h ⁻¹
Aldridge et al. 1995	<i>Chlorella</i>	20	79	mg mg DBM ⁻¹ h ⁻¹
Heath et al. 1995	Natural seston	24	100	mL mg AFDM ⁻¹ h ⁻¹
Lei et al. 1996	Clay with adsorbed bacteria	15	83	mL mg AFDM ⁻¹ h ⁻¹

Filtering rates calculated by different authors were converted to volume of water filtered (mL) per hour per gram of WTM of zebra mussel. n.r., not reported. ind.⁻¹, per individual.

(Stanczykowska 1968, Morton 1971, Reeders and Bij de Vaate 1990, Wisniewski 1990), although some Europeans calculate filtering rate per dry body mass (soft tissue only, DBM) (Kryger and Riisgård 1988). The majority of North American scientists also calculate the filtering rate of zebra mussels per DBM (Aldridge et al. 1995) or per ash-free dry mass (soft tissue only, AFDM) (Fanslow et al. 1995, Heath et al. 1995, Lei et al. 1996).

To compare estimates of filtration calculated by different authors, we converted all available literature data to volume of water filtered (in milliliters) per gram of WTM per hour (Table 4). We used the relationship between shell length and WTM determined by Karatayev (1983) to convert reported zebra mussel shell lengths to WTM. For example, Mikheev and Sorokin (1966) measured the size-specific filtering rate of 9- to 29-mm mussels in short-duration experiments with C_{14} -labeled algae and bacteria. We calculated that the filtering rate in their study ranged from 38 to 160 mL g⁻¹ per hour and averaged 69 mL g⁻¹ per hour.

Comparing all of these various studies (Table 4), we found a relatively narrow range of measured filtering rates for *D. polymorpha* (from 35 to 110 mL g of WTM⁻¹ per hour; average = 58 mL g of WTM⁻¹ per hour), in spite of the fact that these studies were made by different researchers, for different waterbodies, and by different methods. Filtering rates depend on food concentrations (Walz 1978a, Sprung and Rose 1988, Karatayev and Burlakova 1994). According to Sprung and Rose (1988), filtering rates of individual *D. polymorpha* decreased from 290 to 50 mL h⁻¹ when food concentrations (*Chlamydomonas reinhardtii*) increased from 0.1 to 85 cells μ L⁻¹. The extremely high filtering rates (273 mL g of WTM⁻¹ per hour) found by Kryger and Riisgård (1988) may be the result of very low concentrations of algae. Most North American scientists have calculated filtering rates of *D. polymorpha* ranging from 49 to 100 mL g of WTM⁻¹ per hour, averaging 78 mL g of WTM⁻¹ per hour, similar to European results (Table 4).

Common units are essential for cross-study comparisons of filtering rates of *D. polymorpha*. We suggest that the most appropriate units to use are milliliters of filtered water per gram of WTM per hour. WTM varies much less during one growing season than either AFDM or DBM (Karatayev 1983), and WTM can be measured easily and directly, even in the field. We also recommend that field estimates of zebra mussel-filtering rates be calculated as a function of WTM, not density of *D. polymorpha*. Different-sized mussels will filter at different rates; therefore, similar densities of mussels with different size-frequency distributions will have dramatically different filtering rates (Young et al. 1996).

Zebra Mussel as a Biofilter

Because zebra mussels occur in high densities over large areas in lakes, they can filter large volumes of water in relatively short periods of time and deposit vast quantities of pseudofeces on the bottom. In Uchinskoe reservoir (Russia), the population of *D. polymorpha* during the summer could filter the volume of water equivalent to that of the entire waterbody in 45 days (Lvova et al. 1980). Pyalovskoe reservoir (Russia) could be filtered in 20 days (Mikheev 1967a), and the cooling reservoir of the Chernobyl Nuclear Power Station (Ukraine) could be filtered in 5–6 days (Protasov et al. 1983). The time required to filter the entire volume of a variety of lakes in Poland ranged from several days to the entire growing season (Stanczykowska 1977). In two Dutch lakes, the zebra mussel population could filter the volume of water equivalent to that of the entire lake once or twice a month (Reeders et al. 1989).

Water that has been filtered by *D. polymorpha* is almost free of suspended matter (Lvova 1977). Filtered particles that are not ingested are deposited on the bottom as pseudofeces, and postdigested material are deposited as feces. In areas populated with *D. polymorpha* in Uchinskoe reservoir, mussels deposit 1,071 g⁻² of seston annually (Lvova 1977, Lvova 1979). Before the invasion of *D. polymorpha*, the annual deposition of sediment at these sites was only 470 g⁻². The total population of zebra mussels in Pyalovskoe reservoir deposits more than 36,000 tons of suspended matter per year (Mikheev 1967a). In the North-Crimean Canal, *Dreissena* mineralize 786.9 tons of organic matter and deposit 8,872.9 tons in the form of agglutinates per year (Shevtsova and Kharchenko 1981). *D. polymorpha* in the Szczecin Gulf (Poland) filter 53 tons of seston per hour (Wiktor 1969).

D. polymorpha also transform ingested organic matter through digestion. For example, in Volgograd reservoir (Russia), zebra mussels mineralize about 700,000 tons of organic matter in one growing season (Spiridonov 1973). According to Hamburger et al. (1990), 9–18% of the net phytoplankton production is ingested and assimilated by *D. polymorpha* in Lake Esrom (Denmark).

The deposition of large amounts of seston significantly improves the food base for many benthic animals. According to Alimov (1981), in Mikolajskie Lake, the annual dietary requirement for all of the noncarnivorous animals is met by 16% of the seston deposited each year by bivalves. *D. polymorpha* produce 160 of the 164.5 tons of dry seston deposited by all bivalves in this lake.

Before the appearance of *D. polymorpha* in Lukomskoe Lake, benthic filter feeders were capable of filtering the volume equivalent of that of the lake in 15 y, and planktonic filterers could filter that same volume in 5 days, using Kryuchkova's (1989) estimate that zooplankton can filter 120 mL g⁻¹ per day in a eutrophic lake. After *D. polymorpha* invaded Lukomskoe Lake, zooplankton abundance declined, and the time required for the zooplankton to filter the equivalent of the volume of the lake increased to 17 days (Karatayev and Burlakova 1992, Karatayev and Burlakova 1995a). By 1975, because of the presence of *D. polymorpha*, the filtering capacity of benthic invertebrates had increased 320 times, and the equivalent of the volume of the lake could be filtered in 17 days. At present, the benthos can filter this volume in 45 days.

BENTHIC PELAGIC COUPLING

Dreissena shift materials from the pelagic to the benthos by transporting suspended matter from the water column to the benthic community (Lvova-Kachanova 1971, Stanczykowska et al. 1976, Lvova 1977, Lvova 1980, Alimov 1981, Karatayev 1983, Lyakhnovich et al. 1983, Kharchenko and Lyashenko 1985, Karatayev 1988, Karatayev and Lyakhnovich 1988, Lyakhnovich et al. 1988, Shevtsova 1989, Karatayev 1992, Karatayev and Burlakova 1992, Reeders et al. 1993, Karatayev and Burlakova 1995a). A portion of filtered material is metabolized and used for the growth of soft body and shell, and the rest is available to other benthic organisms. Shell materials are permanently removed from the pelagic system and are buried after the death of the zebra mussel.

The movement of seston from the plankton to the benthos induced large changes in all aspects of lake ecosystems after the invasion of *D. polymorpha* (Karatayev 1983, Lyakhnovich et al. 1983, Mitrakhovich et al. 1983, Mitrakhovich 1984, Karatayev 1988, Lyakhnovich et al. 1988, Reeders and Bijde Vaate 1990,

Karatayev 1992, Karatayev and Burlakova 1992, Reeders et al. 1993, Karatayev and Burlakova 1995a). In Lukomskoe lake, early in the invasion of *D. polymorpha* during the growing season, water transparency increased from 1.8 to 4 m, and seston concentrations decreased threefold (Fig. 1). (Lyakhovich et al. 1983, Lyakhovich et al. 1988, Karatayev 1992, Karatayev and Burlakova 1995a). Dissolved organic matter in the water column also decreased. Increased water clarity resulted in an expansion of macrophyte cover (from 6 to 30% of total lake area) due to an increase in the depth at which macrophytes can grow (from 2.5 to 5 m). Subsequent to the invasion of *D. polymorpha*, the biomass of phytoplankton and zooplankton declined more than 10 times, whereas the abundance of zoobenthic organisms increased more than 10 times. The productivity of the fishery doubled, and the composition of the commercial catch is now characterized by an increase in benthophagous fishes that feed on *D. polymorpha* including roach, rudd, white bream, and bream (Karatayev 1983, Lyakhovich et al. 1983, Mitrakhovich et al. 1983, Mitrakhovich 1994, Karatayev 1984, Lyakhovich et al. 1988, Karatayev 1988, Karatayev 1992, Karatayev et al. 1994b, Karatayev and Burlakova 1995a).

After *D. polymorpha* declined in abundance after its initial invasion in Lukomskoe lake, summer transparency decreased to 3 m, but remained above preinvasion levels (1.8–2.0 m) (Karatayev 1992, Karatayev and Burlakova 1995a). Similar patterns were found for phytoplankton and zooplankton; abundance decreased when *D. polymorpha* initially reached very high abundance, but increased after *D. polymorpha* densities declined (Fig. 1). Again, they did not return to their original abundance. The overgrowth of

the lake by macrophytes has also decreased from 30 to 20% of the surface area, but remains higher than that before the zebra mussel invasion (6%) (Karatayev 1992, Karatayev and Burlakova 1995a).

D. polymorpha invaded the Narochnskoe lake system (mesotrophic Narochn lake, eutrophic Myastro lake, and highly eutrophic Batorino lake) in the mid-1980s, after regular studies of these lakes had been conducted for approximately 40 y (Karatayev and Burlakova 1995b). Although morphometry, trophic status, species composition, and density of macrophytes, planktonic, and benthic organisms were different in these lakes (Babitsky 1985, Gavrilov 1985, Kryuchkova 1985, Mikheeva 1985, Ostapenya 1985, Winberg 1985), *D. polymorpha* was associated with similar changes in all three ecosystems (Ostapenya et al. 1993, Ostapenya et al. 1994a, Ostapenya et al. 1994b). After *D. polymorpha* invaded, water transparency increased 1.3–2.4 times, the concentration of seston was reduced 2.3–6.9 times, and the chlorophyll content decreased 2.7–6.9 times. Organic carbon content, BOD₅, primary production, respiration, and biomass of phytoplankton decreased (Ostapenya et al. 1993, Ostapenya et al. 1994a, Ostapenya et al. 1994b). *D. polymorpha* reduce the effects of eutrophication in these lakes. Thus, highly eutrophic Batorino became eutrophic (Ostapenya et al. 1994b), and eutrophic Myastro became slightly eutrophic (Ostapenya et al. 1994a). It appears that *D. polymorpha* can be used to control the negative effects of anthropogenic eutrophication, including increased phytoplankton abundance and decreased water clarity (Karatayev 1983, Karatayev 1984, Karatayev 1988, Karatayev 1992). Some western European scientists have proposed using *D. polymorpha* for biofilters to decrease

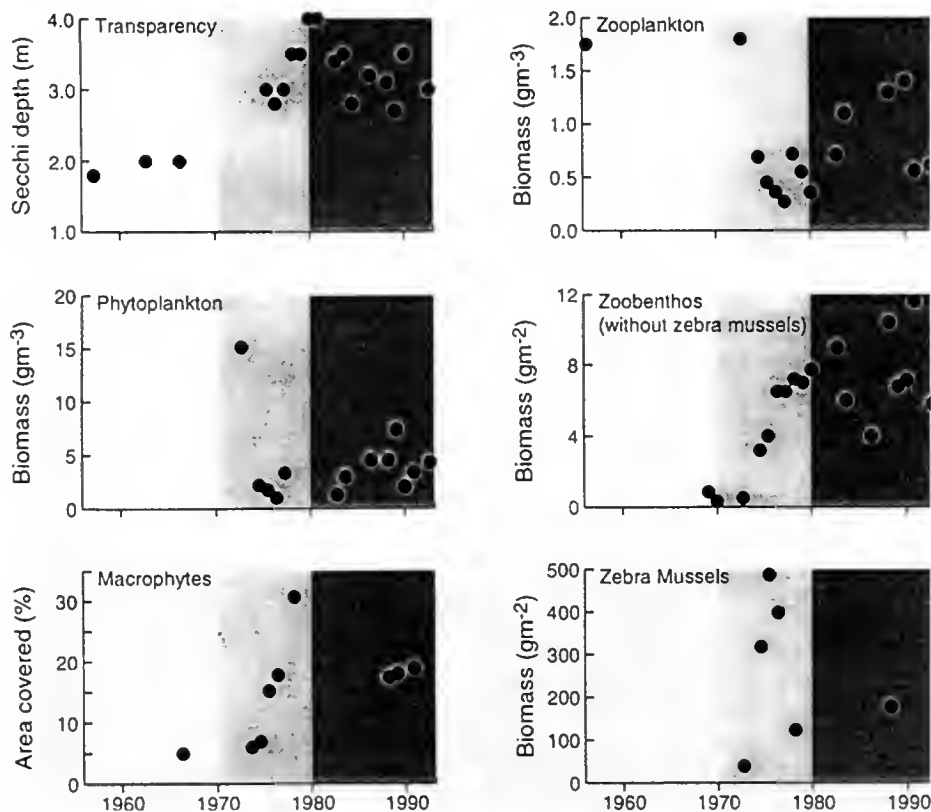


Figure 1. Long-term changes in Lukomskoe lake from before the zebra mussel invasion (before 1970), during the initial invasion (1970–1980), and after there was an established population of zebra mussels (after 1980). Transparency was measured as Secchi depth; phytoplankton and zooplankton are in g of wet biomass m⁻³; macrophytes are in % from the total area of the lake; zoobenthos (without zebra mussels) are in g of wet biomass m⁻²; zebra mussels are in total wet biomass g m⁻².

the effects of anthropogenic eutrophication in lakes (Reeders et al. 1989, Reeders and Bij de Vaate 1990, Noordhuis et al. 1992, Reeders et al. 1993).

North American scientists have reported similar changes in lake ecosystems after the recent appearance of *Dreissena* in the Great Lakes. *Dreissena* have been associated with increases in water transparency (Hebert et al. 1991, Holland 1993, Leach 1993, Fahnenstiel et al. 1995b); increases in benthic algal abundance (Lowe and Pillsbury 1995); expansion of macrophyte beds (Skubinna et al. 1995); decreases in turbidity (Skubinna et al. 1995); decreases in chlorophyll, phytoplankton abundance, and production (Leach 1993, Nichols and Hopkins 1993, Fahnenstiel et al. 1995a, Fahnenstiel et al. 1995b); increases in the density of benthic animals; and changes in benthic community structure (Dermott and Munawar 1993, Griffiths 1993, Stewart and Haynes 1994, Wisenden and Bailey 1995, Botts et al. 1996). In all cases, patterns of zebra mussel effects are similar to those found in FSU and European freshwaters.

INFLUENCE ON FOOD WEBS

The only studies on the effect of zebra mussel on food webs and the energetic balance of the ecosystem in the FSU have been conducted in Lukomskoe lake. Before the invasion of *D. polymorpha*, the total primary production (phytoplankton, 98%; macrophytes, 2%) in this lake was 2,596 kcal m⁻² (Table 5) (Karatayev 1992, Karatayev and Burlakova 1995a). The production of non-predator zooplankton (97%) and zoobenthos (3%) together were 3.7% of total primary production. Fish production was 0.15% of the total primary production (Karatayev 1992, Karatayev and Burlakova 1995a). Lukomskoe lake was similar to many lakes studied

by Bullion and Winberg (1981), and in general, fish production averaged 0.1–0.3% of primary production.

In 1978, after the appearance of *D. polymorpha* in Lukomskoe lake, macrophyte production increased 3.3 times, and phytoplankton production decreased more than 4 times (Karatayev 1992, Karatayev and Burlakova 1995a). Total primary production decreased more than three times. Total production of planktonic and benthic nonpredatory invertebrates declined from 95 to 44 kcal m⁻², and benthic invertebrates increased from 3 to 77% of the total. The production of nonpredatory invertebrates increased to 5.5% of primary production, compared with 3.7% before the invasion of *D. polymorpha*. Fish production increased from 0.15 to 1% of primary production (Karatayev 1992, Karatayev and Burlakova 1995a). This high rate of fish production is typical of commercial fish ponds but is much higher than that of most natural lake communities (Bullion and Winberg 1981). Therefore, subsequent to the appearance of *D. polymorpha*, the conversion of primary production to higher trophic levels increased (Karatayev 1992, Karatayev and Burlakova 1995a).

By 1989, the zebra mussel population in Lukomskoe lake had declined and, in terms of biomass, was relatively stable, and a reanalysis of the biotic balance was compared with that found in 1978 (Karatayev 1992, Karatayev and Burlakova 1995a). Total primary production had increased 13%, and the contribution of macrophytes to the total decreased from 20% in 1978 to 11% in 1989 (Table 5). The total production of nonpredatory zooplankton and benthos was nearly twice that in 1978 as a result of the increased proportion of zooplankton from 23% in 1978 to 45% in 1989. The production of benthic invertebrates increased two times. Fish production remained approximately 1% of primary production, as in 1978 (Karatayev 1992, Karatayev and Burlakova 1995a).

TABLE 5.
Biomass and production of Lukomskoe Lake before and after the appearance of zebra mussels.

Trophic Level	Preinvasion		Postinvasion				P/B
			1978		1989		
	B	P	B	P	B	P	
Primary production							
Phytoplankton	50.9	2,544.5	12.5	624.5	16.1	805.0	50.0
Macrophytes	40.9	51.1	132.8	166.0	81.3	101.6	1.25
Total	91.8	2,595.6	145.3	790.5	97.5	906.6	
Zooplankton filterers							
Crustacea	6.37	92.4	0.70	10.2	3.55	51.5	14.5
Rotifers	0.03	1.4	0.10	4.6	0.36	16.6	46.0
Total	6.40	93.8	0.80	14.8	3.91	68.1	
Nonpredatory zoobenthos	0.38	1.4	3.22	12.2	6.44	24.5	3.8
Zebra mussels	0.00	0.0	27.80	16.7	40.00	24.0	0.6
Zooplankton filterers + nonpredatory zoobenthos	6.78	95.2	31.82	43.7	50.35	116.6	
Predatory zooplankton							
Crustacea	1.22	11.7	0.18	1.7	0.87	8.4	9.6
Rotifers	0.02	1.5	0.05	3.7	0.12	8.8	73.3
Total	1.24	13.2	0.23	5.4	0.99	17.2	
Predatory zoobenthos	0.02	0.1	0.28	1.0	0.56	2.0	3.5
Predatory zooplankton + predatory zoobenthos	1.26	13.3	0.51	6.4	1.55	19.2	
Fish							
Nonpredators	8.75	3.5	17.00	6.8	21.25	8.5	0.4
Predators	1.25	0.5	3.00	1.2	1.25	0.5	0.4

Biomass (B) and production (P) are given in kcal m⁻² (from Karatayev and Burlakova 1995a).

CHANGES IN FISHES

Twenty-seven fish species in Europe and 14 species in North America are known to consume *Dreissena* (Molloy et al. 1997 and references therein). Common carp (*Cyprinus carpio*), pumpkinseed (*Lepomis gibbosus*), and round goby (*Neogobius melanostomus*) have been field documented as predators on both continents. Another 13 North American species have been mentioned in the literature as potential predators (Molloy et al. 1997).

Dreissena are readily eaten by fish in the North Caspian Sea, where approximately 90% of the annual production of mussels (130,000 tonnes) are eaten by fish (Yablonskaya 1985). The roach is the most prominent consumer of *Dreissena* in European freshwaters (Pliszka 1953, Grigorash 1963, Mikheev 1963, Filuk and Zmudzinski 1965, Prejs 1976, Lvova 1977, Stanczykowska 1987, Karatayev et al. 1994b, and others). *D. polymorpha* comprise from 95 to 100% of the diet of roach larger than 18 cm in a number of Polish lakes (Pliszka 1953, Prejs 1976, Stanczykowska 1987). Since the *D. polymorpha* invasion in the reservoirs of the Volga cascade (Russia), a new population of mussel-eating roach has developed, characterized by very high individual growth rates and large body size (Poddubnyi 1966). In Uchinskoe reservoir, benthophages fish (mainly roach) eat approximately 80% of the yearly production of *D. polymorpha* under 15 mm (Lvova 1977) and were the most abundant fish in this reservoir (Spanowskaya 1963). Before the zebra mussel invasion, the growth rate of young-of-year roach was almost the same as that in other reservoirs. However, when the roach were able to eat *D. polymorpha*, their growth rate and lipid content significantly increased and exceeded those of roach in reservoirs without *D. polymorpha* (Lyagina and Spanowskaya 1963).

Freshwater drum (*Aplodinotus grunniens*) are active consumers of *Dreissena* in North America (French 1993, French and Bur 1993). Another recent invader to the Great Lakes, the round goby, also feeds on *Dreissena* (Jude et al. 1995). Therefore, the presence of *Dreissena* may enhance the spread of this second invader.

The effect of zebra mussels on fish may be direct or indirect. The direction and intensity of these effects are dependent on the feeding method of the majority of the fish in a waterbody. In general, we may expect an enhancement of all benthic feeding fishes, even those that do not feed on *Dreissena*, because *Dreissena* increase the biomass of other benthic invertebrates (Kharchenko and Protasov 1981, Karatayev 1983, Lyakhovich et al. 1983, Lyakhovich et al. 1988, Karatayev 1992, Karatayev and Burlakova 1992, Dermott and Munawar 1993, Griffiths 1993, Stewart and Haynes 1994, Karatayev and Burlakova 1995a, and others). We found no documented effects of zebra mussel presence on planktivorous fish.

GENERAL FINDINGS

The measured effects of *D. polymorpha* on a community will depend on the amount of time since the appearance of zebra mussels, the density of zebra mussels, and the potential species pool of the community. Usually, there is a lag time between when zebra mussels first invade a new waterbody and when they are abundant enough to detect and have ecologically relevant effects. Five to 10 y after initial invasion, *Dreissena* generally rapidly increase in population size (Sebestyen 1937, Berg 1938, Zhadin 1946, Zhuravel 1951, Ovchinnikov 1954, Kondratiev 1958, Lyakhov 1961, Lyakhov 1962, Lvova 1977, Lvova 1980, Karatayev 1983, Karatayev et al. 1994a, Karatayev and Burlakova 1995b). For

example, zebra mussels invaded Naroč in the mid-1980s and were at relatively low densities for several years. In 1990, the entire lake average densities were 7.2 m⁻², but rapidly increased to 798 m⁻² in 1993 (Karatayev and Burlakova 1995b). The first zebra mussel was found in Uchinskoe reservoir in 1945, but biomass did not reach a maximum until 1957. Although mussel densities remained similar for several years, the size structure of the population changed, such that total biomass declined and was then relatively stable for 10 y. In 1972, the water flow through the Uchinskoe reservoir was increased three times, increasing local food availability, and the average biomass of zebra mussels increased.

Although *Dreissena* take several years to colonize all regions of lakes they invade, they spread very rapidly through canals of moving water. The North-Crimean Canal (Ukraine) was built in 1966 (Kharchenko and Protasov 1979), and by 1967–1969, the number of *Dreissena* in some areas was 1,782 m⁻² and the biomass was 96.4 g m⁻² (Kaftannikova 1975). By 1984, *Dreissena* had colonized the entire Dnieper-Donbass Canal, which was opened in 1983 (Lyashenko and Kharchenko 1988).

The general pattern that has emerged from long-term studies in Europe, including the FSU, is that initial populations of zebra mussels grow to very high densities, but because of density-dependent processes, total sustainable biomass declines as the system changes and densities well below the maximum achieved persist (Sebestyen 1937, Zhadin 1946, Zhuravel 1951, Lyakhov 1962, Lvova 1977, Walz 1978b, Lvova, 1980, Karatayev 1983, Karatayev and Burlakova 1995a). However, all populations of zebra mussels do not stabilize and can change widely (Ramcharan et al. 1992). On the basis of observations from 1959 to 1988 on 12 Mazurian lakes (Poland) with established zebra mussel populations, Stanczykowska and Lewandowski (1993) found 4 lakes with stable zebra mussel populations, 4 lakes with unstable populations, and 4 lakes with populations that had declined. The most dramatic changes were found in Lake Mikolajskie. From 1959 to 1960, the average density of zebra mussels in this lake decreased from 2,200 to less than 50 m⁻². In 1976, the population increased to more than 2,000 m⁻² and then again dramatically declined (Stanczykowska and Lewandowski 1993).

We hypothesize that early in an invasion, when population levels are climbing and are high, *D. polymorpha* will have their largest effects on communities, and most of the effects will be direct. The effects of *D. polymorpha* on communities after the initial stages of invasion are much less predictable, and much more likely to be caused by indirect effects through changes in the ecosystem. The filtering activity of zebra mussels increases water transparency and organic matter mineralization (Table 6). *D. polymorpha* reduce the concentration of seston in the water column and reduce densities and the production of phytoplankton. Improved water transparency increases macrophyte biomass and coverage as macrophytes grow deeper in the lake. Increased macrophyte abundance may act as a barrier hindering the influx of allochthonic nutrients used by phytoplankton. Increased light penetration is also likely to stimulate an increase in periphyton. Only scattered data are available on bacterioplankton and suggest an increase in abundance with zebra mussels.

In the presence of *Dreissena*, the abundance of zooplankton is reduced and is accompanied by structural changes in the plankton community. The numbers, biomass, and production of benthic invertebrates are increased, and the taxonomic and trophic structure of benthic animals changes.

TABLE 6.
The influence of zebra mussels on freshwater
ecosystem characteristics.

Parameter	Change With Zebra Mussels	References
Transparency	Increase 1.5, 2, and >2×	Stanczykowska 1968, Lvova-Kachanova 1971, Stanczykowska 1977, Lvova 1979, Karatayev 1983, Karatayev 1984, Karatayev 1992, Griffiths 1993, Holland 1993, Reeders et al. 1993, Fahnenstiel et al. 1995b
Seston	Decrease 1.5–10×	Lvova et al. 1980, Kharchenko and Lyashenko 1985, Leach 1993, Reeders et al. 1993, Ostapenya et al. 1994a, Ostapenya et al. 1994b, Karatayev and Burlakova 1995a
Organic matter	Decrease. Increase of organic matter mineralization	Kharchenko and Lyashenko 1985, Shevtsova 1989, Ostapenya et al. 1994a, Ostapenya et al. 1994b, Karatayev and Burlakova 1995a
BOD ₅	Decrease 1.5×	Kharchenko and Lyashenko 1985, Ostapenya et al. 1994a, Ostapenya et al. 1994b
Phytoplankton	Decrease 1.5–4× quantity, chlorophyll	Lyakhnovich et al. 1988, Karatayev 1992, Noordhuis et al. 1992, Holland 1993, Leach 1993, Nichols and Hopkins. 1993, Reeders et al. 1993, Ostapenya et al. 1994a, Ostapenya et al. 1994b, Karatayev and Burlakova 1995a
Primary production of phytoplankton	Decrease 2–4×	Ostapenya et al. 1994a, Ostapenya et al. 1994b, Fahnenstiel et al. 1995a, Fahnenstiel et al. 1995b
Bacterioplankton	Slightly increase numbers	Ostapenya et al. 1994a, Ostapenya et al. 1994b
Macrophytes	Increase biomass and overgrowth rate	Lyakhnovich et al. 1988, Reeders and Bij de Vaate 1990, Griffiths 1993, Skubinna et al. 1995
Phytoperiphyton and phytobenthos	Increase quantity, chlorophyll and primary production	Lowe and Pillsbury 1995
Zooplankton	Decrease quantity, structural changes in community	Lyakhnovich et al. 1983, Mitrakovich et al. 1983, Mitrakhovich 1984, Shevtsova et al. 1986, Karatayev et al. 1994a
Zoobenthos	Increase quantity, changes in taxonomic and trophic structures	Dusoge 1966, Wiktor 1969, Kharchenko and Protasov 1981, Karatayev 1983, Karatayev et al. 1983, Kharchenko 1989, Karatayev 1992, Karatayev and Burlakova 1992, Griffiths 1993, Dermott and Munawar 1993, Stewart and Haynes 1994, Winseden and Bailey 1994, Karatayev and Burlakova 1995a, Botts et al. 1996
Fishes	Increase quantity of benthophages	Karatayev 1983, Lyakhnovich et al. 1988, Karatayev 1992, Karatayev and Burlakova 1995a

By comparing the effect impact of *D. polymorpha* across different waterbodies located in a variety of geographical areas in the Old World, we found similar changes in native ecosystems. Would we expect the same to be true in North America? North American freshwaters may be influenced more by *D. polymorpha* than the waterbodies of Eurasia if the lack of evolutionary history with dreissenids is important, or if dreissenids have different effects on very large lakes such as the Laurentian Great Lakes. In order to determine if North America and Europe are different, we must have comparable, similarly collected data. Differences in methodology and what data are collected have hindered our ability to compare current North American information with that from Europe. The types of information that would be most useful are: (1)

Estimates of whole-lake densities of *Dreissena* with either size-frequency distributions or WTM. Information should be collected using randomized survey techniques to ensure that all habitat types are included, not just those with high densities of *Dreissena*. (2) Before and after invasion measures of the biomass of phytoplankton, zooplankton, fish, macrophytes, periphyton, and benthic invertebrates, especially bivalves. In addition, the abundance of ecologically important groups (predators, gatherers, filterers, etc.) before and after invasion should be determined. With these data, we will not only be able to establish the general effects of *Dreissena* on freshwater ecosystems, but we will be able to test whether *Dreissena* have different effects on large versus small lakes and on different continents.

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ALTERNATIVE REBUILDING STRATEGIES FOR THE RED KING CRAB *PARALITHODES CAMTSCHATICUS* FISHERY IN BRISTOL BAY, ALASKA

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ABSTRACT The abundance of Bristol Bay red king crab *Paralithodes camtschaticus* has fallen to historically low levels in recent years. Concerns about depressed spawning stock levels and economic hardships associated with fishery closures in 1994 and 1995 prompted reevaluation of the status quo harvest strategy and investigation of alternative strategies to rebuild the stock. Using a length-based model initialized with the 1994 population abundance, we simulated future effects of seven alternative rebuilding strategies on this stock. Strategies ranged from the status quo strategy through increasingly restrictive harvest strategies culminating in complete fishery closure until the stock is rebuilt. Statistics on catch, variation in catch, effective spawning biomass, probability of rebuilding, probability of fishery closure, and present exvessel value were collected for comparisons. Sensitivities of the harvest strategies to natural mortality, handling mortality, stock-recruitment relationship, and measurement error were examined. Reducing the status quo harvest rate greatly shortened rebuilding time and enhanced long-term catch. The most conservative strategy achieved a 50% probability of rebuilding the stock to a target 25,000 t of effective spawning biomass in 12 y. In terms of catch and present exvessel value, the status quo strategy performed best among the seven strategies over a short-term (≤ 20 y) planning horizon, whereas strategies with a mature male harvest rate of 50–75% of the status quo level performed best over longer planning horizons. Scenarios with lower natural mortality, higher handling mortality, or a more density-dependent stock-recruitment relationship favor the implementation of more conservative strategies. Our analysis of population and fishery dynamics leads us to recommend a 25–50% reduction in harvest rate to rebuild this depressed stock.

KEY WORDS: Red king crab, *Paralithodes camtschaticus*, rebuilding strategies, fishery management, Alaska

INTRODUCTION

Red king crab (RKC), *Paralithodes camtschaticus*, in Bristol Bay, AK, once supported one of the most important fisheries in the United States. A peak catch of 59,000 t occurred in 1980, and thereafter, stock abundance declined rapidly (Otto 1990). No fishing was allowed in 1983, but it resumed in 1984. However, catch and stock abundance remained at low levels. Fishing was prohibited again in 1994, at which time, spawning biomass was <10% of the highest estimated value, and recruitment to the mature population was the lowest ever documented during the past three decades (Zheng et al. 1995a, Zheng et al. 1995b).

The fishery was historically managed by use of a variable legal male harvest rate strategy set from a look-up-table according to relative population size and prerecruitment and postrecruitment abundance levels (Otto 1985). This strategy was revised in 1990, with a constant harvest rate set at 20% of the mature male population, provided that no more than 60% of the legal male population is harvested (Pengilly and Schmidt 1995). Further, no fishing is allowed when the population of mature females is at or below a threshold of 8.4 million crabs. In this study, we term this suite of management measures the “status quo strategy.” Additional management measures include area closures, gear restrictions, size limit (legal crabs only, ≥ 135 mm carapace length [CL]), sex restriction (males only), and seasonal closures (no fishing during spring molting and mating period). Poor success in maintaining a healthy stock over the years precipitated reexamination of the status quo harvest strategy (Kruse 1993). The current depressed population abundance and fishery closure underscore the need to investigate rebuilding strategies.

The purpose of this study is to analyze seven alternative rebuilding strategies for Bristol Bay RKC with computer simulations, based on a length-based population model (Zheng et al.

1995b). The alternative strategies range from conservative (no fishing until the stock has been rebuilt to a target level) to liberal (the status quo strategy). On the basis of our findings, we recommend remedial management actions to improve chances of stock recovery. In a separate study (Zheng et al. in press), we examined optimal long-term harvest strategies once the stock is rebuilt.

METHODS

Population Model and Parameters

The length-based population model constructed by Zheng et al. (1995a and 1995b) was used in this study and is summarized in the Appendix. We set the minimum CL at 95 mm for males and 90 mm for females and simulated crab abundance using length class intervals of 5 mm. The last length class included all crabs with lengths ≥ 169 mm for males and ≥ 140 mm for females. Population parameters were obtained from Zheng et al. (1995b) and are summarized in Table 1. Population abundances were simulated for June each year, after crabs have generally completed annual molting and mating. Because fishing has occurred during the first 2 wk of November each year since 1990, we used a lag of about 4.8 mo, or 0.4 y, between abundance assessment and the November fishery in our simulations.

A constant natural mortality was used in our simulations because the alternative strategies were affected within a relatively short time span and because potential future shifts in natural mortality are not predictable. The natural mortality of Bristol Bay RKC changed over time (Zheng et al. 1995b), so we computed long-term means for male and female crabs (Table 1) as weighted averages of annual natural mortalities from 1972 to 1994, estimated by Zheng et al. (1995b). Years of high natural mortality are rare for Bristol Bay RKC, and the current natural mortality is the lowest observed. Therefore, a 50% weight was assigned to high

TABLE 1.
Population parameters for a length-based model of Bristol Bay RKC (Zheng et al. 1995b).

Mid-CL (mm)	Weight (Kg)		Initial Abundance (mill)			Maximum Female Mates/Male	Male Molting Probability
			Male		Female		
	Male	Female	New*	Old*			
92.5		0.564			0.132	0.0	
97.5	0.696	0.634	0.247	0.040	0.414	0.0	0.980
102.5	0.815	0.709	0.252	0.062	0.814	0.0	0.969
107.5	0.948	0.789	0.323	0.113	1.109	0.0	0.953
112.5	1.094	0.873	0.545	0.253	1.186	0.0	0.928
117.5	1.256	0.962	0.619	0.367	1.086	0.0	0.893
122.5	1.432	1.056	0.699	0.446	1.009	1.0	0.842
127.5	1.625	1.155	0.855	0.536	1.014	1.2	0.773
132.5	1.835	1.258	0.872	0.594	0.896	1.4	0.686
137.5	2.063	1.367	0.736	0.468	0.627	1.6	0.583
142.5	2.310	1.480	0.595	0.367	0.508	1.8	0.472
147.5	2.576		0.446	0.410		2.1	0.364
152.5	2.862		0.275	0.320		2.4	0.264
157.5	3.169		0.156	0.222		2.7	0.190
162.5+	3.498		0.231	0.412		3.0	0.131

Growth Parameter			Natural Mortality		
	Male	Female		Male	Female
a	13.140	16.490	Mean	0.29	0.47
b	0.018	-0.097	Low	0.23	0.32
β	0.519	0.931	High	0.40	0.62

S-R Relationship						
	Ricker Model				Proportion by Length	
	General	Autocorrelated	Combined		Male	Female
r1	2.332	1.000	1.880	α _r	54.115	303.325
r2	-11.403	0.523	-7.396	β _r	1.885	0.313
r3	6.49E-5	2.05E-5	4.86E-5			
k	7.000	7.000	7.000			
a1	0.000	0.600	0.340			
σ	0.700	0.700	0.700			

natural mortality levels from 1980 to 1984 for males and from 1981 to 1984 for females, and a 100% weight was given to those in remaining years. To examine the sensitivity of the alternative strategies to levels of natural mortality, we compared evaluation criteria for low natural mortality, represented by the lowest estimated annual natural mortality, and high natural mortality, estimated by equally weighting the average of annual natural mortalities from 1972 to 1994 (Table 1). In our opinion, these low and high natural mortalities reasonably represent extreme ranges of mean natural mortality to be expected over the rebuilding period.

To estimate handling mortality, we assumed that catchability of sublegal male and mature female crabs is 50% of that for legal male crabs, based on overall observed bycatch rates for the Bristol Bay RKC fishery in 1990 and 1991 (Beers 1991, Beers 1992). A 20% handling mortality rate (Zheng et al. in press) for mature female (>89 mm CL) and sublegal male (95–134 mm CL) crabs that are caught and returned to the sea was assumed. To investigate the sensitivity of results to handling mortality, we also simulated scenarios with 0 and 50% handling mortality rates.

The bycatch limit of RKC in the eastern Bering Sea groundfish fisheries from 1989 to 1996 was 200,000 crabs per year (Witherell

and Harrington 1996). Therefore, we deducted 200,000 RKC lost to the groundfish fisheries each year using equal catchability for legal and sublegal male and mature female crabs.

Survey measurement error was assumed to follow a lognormal distribution. True effective spawning biomass and legal abundance by simulation were multiplied by a measurement error to mimic "estimated" values for each year. The lognormal measurement errors were simulated with a standard deviation of 0.2 and mean of zero. Standard deviations of 0 and 0.5 were used for sensitivity studies. To prevent extremely large errors in estimated values of abundance, both ends of the measurement-error distribution were truncated to fall within its 98% confidence limits.

Stock-recruitment (S-R) data for Bristol Bay RKC were obtained from Zheng et al. (1995b) and used to fit a four-parameter Ricker curve that combined two special cases of the general S-R model: a general Ricker curve that attributes change in recruitment to effective spawning biomass ($r_1 > 1$ and $a_1 = 0$; see Appendix) and an autocorrelated Ricker curve that emphasizes recruitment change due to environmental causes ($r_1 = 1$ and $a_1 > 0$). The combined curve fit the data well ($R^2 = 0.62$, $df = 15$; Fig. 1) and was used to conduct our simulations. Because the combined curve

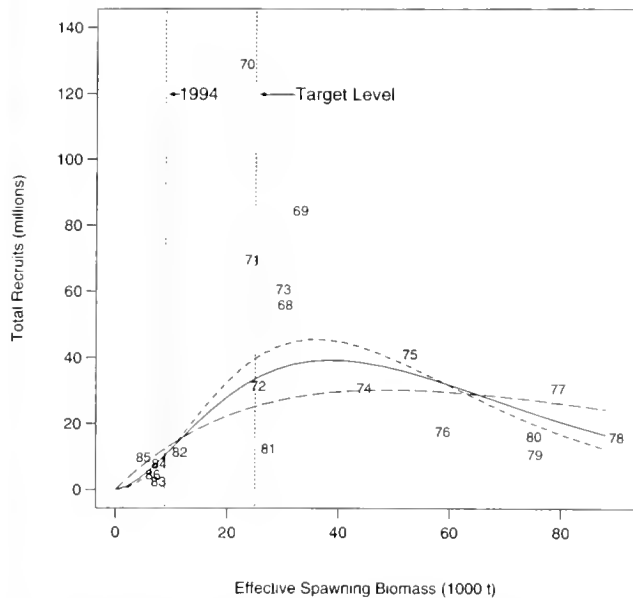


Figure 1. Relationship between total recruits at age 6.2 (i.e., 7-y time lag) and effective spawning biomass for Bristol Bay RKC. Numbers refer to brood year. The solid line is the combined Ricker curve, the short dashed line is the general Ricker curve, and the long dashed line is the autocorrelated Ricker curve. Dotted lines indicate the 1994 level of 8,800 t and the rebuilding target level of 25,000 t.

synthesizes two different, but potentially valid, interpretations of the data, the sensitivity of the strategies to the other two curves was examined. The sex ratio of recruits was assumed to be 1:1.

Alternative Strategies

In this study, we examined seven alternative strategies that we believed most likely to be implemented to rebuild the stock (Table 2) rather than estimating an optimal rebuilding strategy. Optimal long-term harvest strategies after the population has been rebuilt were investigated by Zheng et al. (1997). An optimal rebuilding strategy can theoretically provide the best results for a given objective function, but it may not be practical. For example, the fishery is not manageable with a small catch quota because of the large number of fishing vessels participating in the fishery and the

time lag between catch and catch reporting. Therefore, mature male harvest rates for the seven alternative rebuilding strategies were set to either 0 or $\geq 10\%$.

A rebuilding target for all strategies was set equal to an effective spawning biomass of 25,000 t. This is an intermediate level of biomass above which strong recruitment occurred with a high frequency in the past (Fig. 1). Among the seven alternative rebuilding strategies, the most conservative strategy (Strategy 3), in terms of harvest restraint, does not allow fishing until the population reaches the target level. Status quo (Strategy 1) is the most liberal. The five other strategies have stepped levels of threshold and mature male harvest rate, intermediate to Strategies 1 and 3, when the stock is above threshold but below the target level. Our primary interest was the response of evaluation criteria during rebuilding. Therefore, once the goal of the stock reaching the target biomass was achieved, we used the status quo mature male harvest rate (0.2) for all strategies. Thus, different results from different strategies arise mainly during rebuilding periods.

Simulations

The seven strategies were compared through computer simulations of population dynamics with a standard set of population parameters. Simulations were initialized with the population abundance in 1994 (Table 1) and effective spawning biomass from 1987 to 1994 (Fig. 1). Simulated time horizon was set at 50 y. Year Number 1 corresponded to 1995 and Year Number 50 to 2045. Each scenario was replicated 500 times to ensure relative stability of statistics collected for comparisons. Identical seeds for random number generators were used for all scenarios to compare different strategies under the same environmental conditions.

We examined the sensitivity of each strategy to changes in natural mortality, handling mortality, S-R relationship, and measurement error. The standard set of population parameters was used in each sensitivity analysis, except that the parameter under consideration was assigned one of two opposite and extreme values.

To evaluate the strategies, statistics were collected on effective spawning biomass, rebuilding probability, probability of being below the initial biomass, probabilities of fishery closure and continuous fishery closure, catch, and present exvessel value of the fishery. Rebuilding probability is defined as the proportion of replicates with effective spawning biomass reaching the target level in

TABLE 2.
Seven alternative rebuilding strategies for Bristol Bay RKC.

Specification	Strategy						
	1	2	3	4	5	6	7
Threshold							
ESB (1,000 t)	6.6	11.0	25.0	6.6	6.6	11.0	11.0
Mature Females (1,000,000 crabs)	8.4	8.4	8.4	8.4	8.4	8.4	8.4
Mature male harvest rate above threshold & below target biomass	0.2	0.2	N/A	0.1	0.15	0.1	0.15
Mature male harvest rate at or above target biomass	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Target biomass is 25,000 t of effective spawning biomass (ESB), and maximum legal male harvest rate is 60% for all rebuilding strategies. N/A, not applicable.

a given year. Probabilities of fishery closure and continuous fishery closure are denoted as the proportions of replicates with estimated effective spawning biomass and mature female abundance below threshold (Table 2), so that the fishery is prohibited for a given year (fishery closure) and through a given year (continuous fishery closure starting from Year 1).

The present exvessel value E_i in Year Number i was calculated as

$$E_i = \frac{C_i X_i}{(1 + d)^i} \quad (1)$$

where C_i is total catch in Year Number i in millions of kilograms, d is the discount rate set at 2 or 7%, and X_i is the price per unit of catch in Year Number i expressed in U.S. dollars per kilogram of crabs. Price, X_i , is a linear function of total catch and time:

$$X_i = a + bC_i + ci. \quad (2)$$

where a , b , and c are constants estimated as 1.1961, -0.0536, and 0.5003 by linear regression ($R^2 = 0.83$, $df = 16$) of fishery data from 1973 to 1992, in which we set $i = 1$ for 1973.

Price is also affected by RKC catches elsewhere in the north Pacific Ocean and by the exchange rate between the U.S. dollar and the Japanese yen. It is difficult to model future effects of these two factors. If the trends of these two factors during the simulated years change dramatically from the past 20 y, our price model will not accurately predict the true values.

Evaluation Criteria

We used three criteria common in harvest strategy analyses to evaluate the seven strategies: maximum catch, present exvessel value, and equal tradeoff between the two (Hightower and Grossman 1987, Quinn et al. 1990, Ianelli and Heifetz 1995). The equal tradeoff criterion maximizes catch minus its standard deviation to provide a tradeoff between long-term yield and variation in yield. Besides these criteria, we also examined the probability of rebuilding and loss of harvest opportunity under each strategy in terms of fishery closure.

One important aspect of evaluating a given rebuilding strategy is to examine the tradeoff between the short-term loss, in terms of reduced catch, value, or harvest opportunity, and the long-term gain, in terms of how much the strategy will pay off in the long term. To examine tradeoffs, we compared alternative strategies with the above criteria over different planning horizons (2–50 y). We also computed the number of years required for the six new strategies to outperform the status quo strategy.

RESULTS

Effective Spawning Biomass and Probabilities of Rebuilding and Fishery Closure

Effective spawning biomass generally increased over time for all seven strategies (Fig. 2). The effective spawning biomass for the status quo strategy (Strategy 1) was much lower than those for the other six strategies. The highest effective spawning biomass resulted from the most conservative strategy (Strategy 3), followed by Strategies 6, 4, 7, 5, 2, and 1.

Distributions of effective spawning biomass in a given year skewed toward large biomass (Fig. 3), i.e., densities of the distributions were concentrated at the lower end of effective spawning biomass. The modes of the distributions gradually shifted to higher

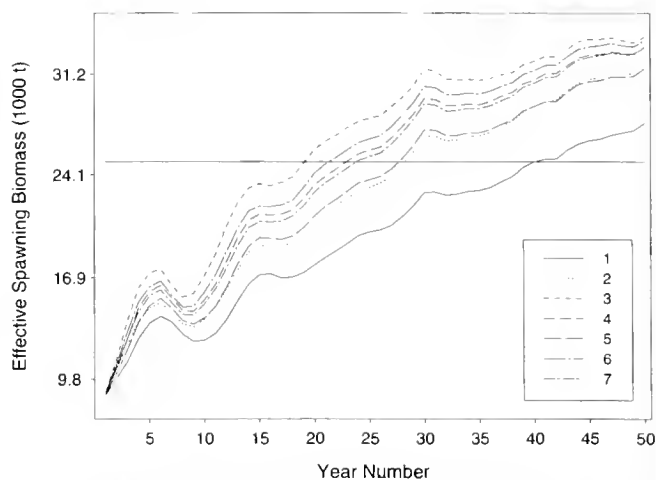


Figure 2. Average annual effective spawning biomass from 500 replicates for seven rebuilding strategies by year. The horizontal line denotes the effective spawning biomass target, and numbers refer to rebuilding strategy.

levels of effective spawning biomass over time. Strategy 3 shifted the fastest, whereas the status quo strategy (Strategy 1) shifted the slowest. Ranges of effective spawning biomass were broad, spanning values well below 10,000 t to above 80,000 t for all strategies in Year Number 50.

As expected, the most conservative strategy (Strategy 3) had

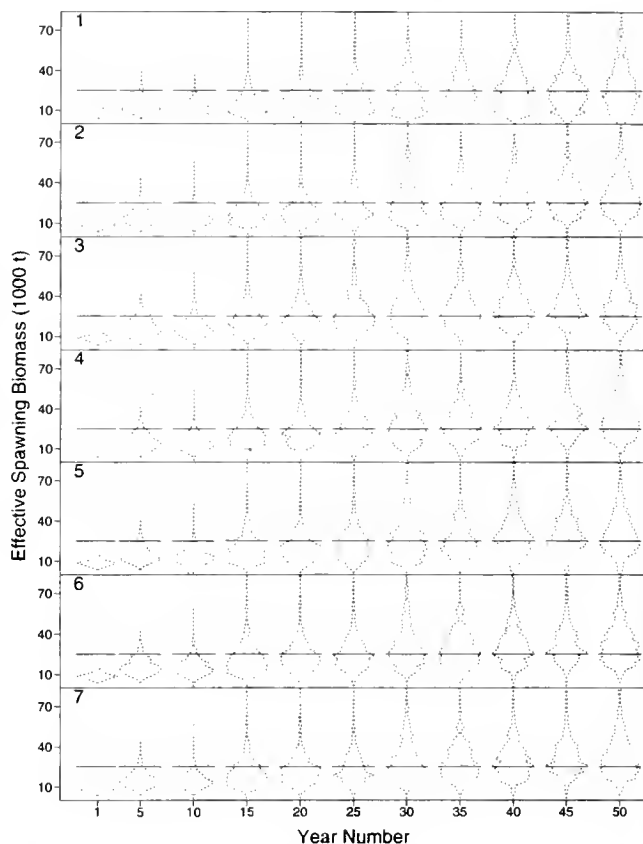


Figure 3. Empirical distributions of annual effective spawning biomass from 500 replicates by year for seven rebuilding strategies. Numbers refer to rebuilding strategy, and short horizontal lines denote the effective spawning biomass target of 25,000 t.

the highest probability of fishery closure during the first 20 y of the planning horizon (Fig. 4). Strategies with relatively high threshold. Strategies 2, 6, and 7, also caused high probabilities of fishery closure during the early period of the planning horizon. After 35 y, the most liberal strategy, status quo (Strategy 1), had the highest probability of fishery closure. Overall, after 25 y, all strategies had a <13% probability of fishery closure. Strategy 4, with a low threshold and a low harvest rate, had the lowest probability of fishery closure for all planning horizons among the seven strategies considered (Fig. 4).

Probabilities of continuous fishery closures depended on threshold levels and declined dramatically over time (Fig. 4). The probabilities were highest for Strategy 3 with the highest threshold level, intermediate for Strategies 2, 6, and 7, and lowest for Strategies 1, 4, and 5 with the lowest level of threshold. After Year Number 5, probabilities of continuous fishery closures were much lower than probabilities of fishery closure (Fig. 4).

One of the most important measures of rebuilding is the probability of rebuilding to the target level. Rebuilding probabilities increased over time for all seven strategies (Fig. 5). The highest ranked strategy, in terms of high rebuilding probability, was Strategy 3, followed by Strategies 6, 4, 7, 5, 2, and 1. This ranking is the same as that found for effective spawning biomass. During the second half of the planning horizon, all six new strategies had a >10% higher probability of rebuilding than the status quo strategy (Fig. 5).

Rebuilding times for all strategies were quite long (Table 3). For a given rebuilding probability, the most conservative harvest

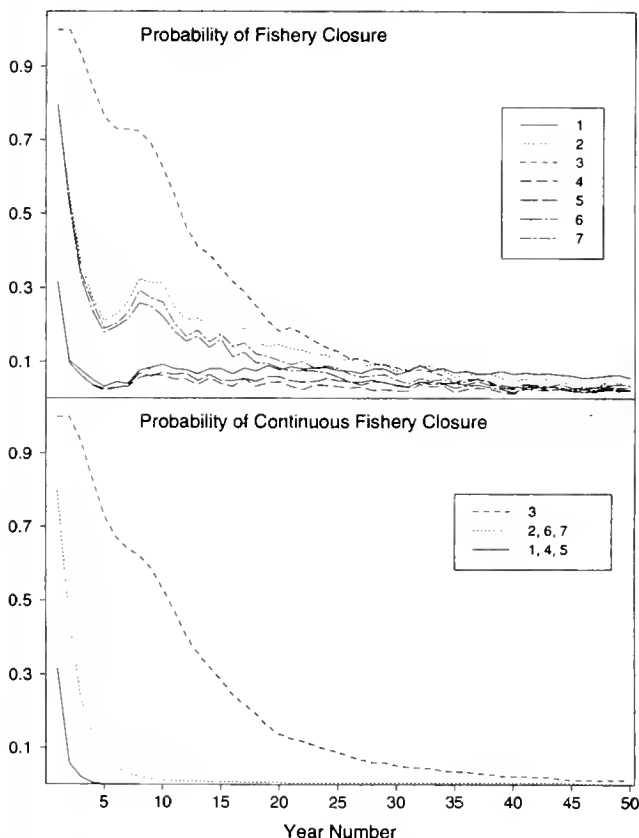


Figure 4. Probabilities of fishery closure by year (upper plot) and continuous fishery closure through year (lower plot) for seven rebuilding strategies. Numbers refer to rebuilding strategy.

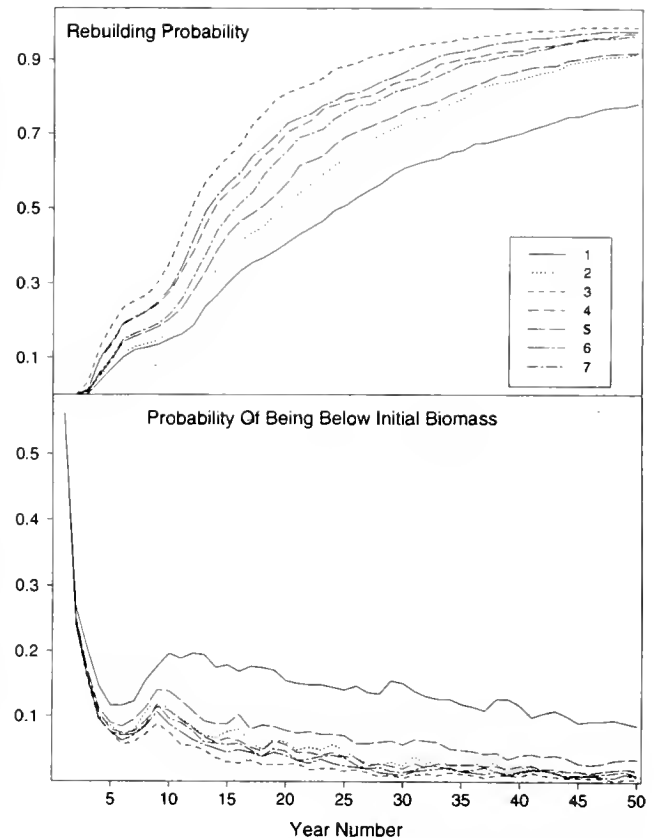


Figure 5. Probabilities of rebuilding to the effective spawning biomass target of 25,000 t (upper plot) and of being below the 1994 initial effective spawning biomass of 8,800 t (lower plot) for seven rebuilding strategies by year. Numbers refer to rebuilding strategy.

strategy (Strategy 3) required the least number of years to rebuild the population: 12 y to rebuild to the target level with a 50% probability and 27 y to rebuild with a 90% probability. The status quo strategy, on the other hand, took 25 y to rebuild with a 50% probability and >50 y to rebuild with a 90% probability. Increasing the threshold from 6,600 to 11,000 t (Strategies 1 versus 2, 4 versus 6, and 5 versus 7) slightly shortened the rebuilding times for a given probability of rebuilding.

Probabilities that the population is below an initial effective spawning biomass of 8,800 t were >50% in Year Number 1 for all strategies but decreased quickly over time (Fig. 5). This probability was above 10% for the status quo strategy through the 50-y planning horizon and was much higher than those for the other strategies. As a comparison, Strategy 3 had the lowest probability, which was below 3% after Year Number 25, but not much different than probabilities for Strategies 2, 4, 6, and 7.

Catch and Present Exvessel Value

Expected annual catches generally increased over time for all strategies (Fig. 6). The status quo strategy produced the highest catches during the first 10 y and the lowest catches after 13 y. In contrast, Strategy 3 had the lowest catches for the first 13 y and the highest catches after 17 y. Generally, strategies with a low harvest rate and a high threshold resulted in low catches at the beginning of simulations and high catches during the later part of the planning horizon. Mean catches over the planning horizon illustrate

TABLE 3.

Minimum years required to rebuild effective spawning biomass to 25,000 t under a given probability of rebuilding for Bristol Bay RKC.

Probability (%)	Strategy						
	1	2	3	4	5	6	7
50	25	20	12	14	18	14	16
60	30	24	14	17	21	17	19
70	40	29	17	20	26	20	22
80	>50	36	20	27	34	25	29
90	>50	46	27	35	45	33	38
95	>50	>50	34	45	>50	40	45
99	>50	>50	>50	>50	>50	>50	>50

that the six new strategies can be expected to take 21–28 y to outperform the status quo strategy (Fig. 6).

Standard deviations of mean catches over the planning horizon for all strategies increased as the planning horizon became longer, but at a slower rate than mean catches (Fig. 6). The standard deviations were smaller than corresponding mean catches for all strategies. Strategy 3 produced the highest catch variation over a given planning horizon when the planning horizon was >5 y. In contrast, the status quo strategy had the lowest catch variation over the planning horizon after the first 10 y (Fig. 6).

The present exvessel values depend on assumed discount rate, which is somewhat subjective (Fig. 7). With a 2% discount rate,

expected mean present exvessel values increased over the planning horizon, and the conservative harvest strategies (3, 4, 6, and 7) outperformed the liberal harvest strategies (1, 2, and 5) over a planning horizon >35 y. With a 7% discount rate, expected mean present exvessel values as a function of planning horizon increased to a maximum value and then declined for each strategy. The status quo strategy had the highest value at the beginning of the planning horizon and underperformed other strategies over a long planning horizon, reflecting the catch pattern. A high discount rate (7%) diminished the benefits of conservative strategies.

The performance of the six new strategies compared with that of the status quo strategy depended on the objective function or criterion. For the maximum catch criterion, it took 21–28 y for

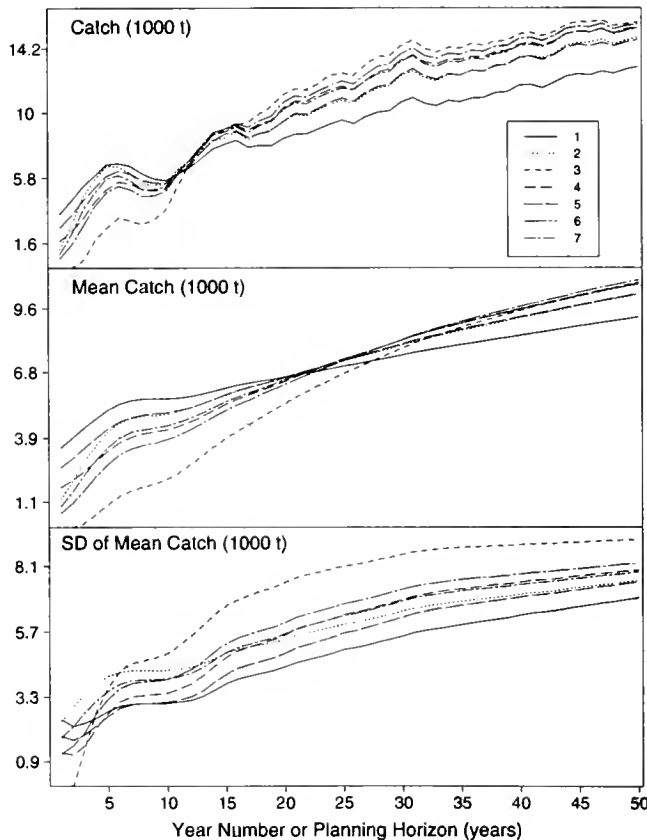


Figure 6. Average annual catch from 500 replicates for seven rebuilding strategies by year (upper plot), average catch over planning horizon (middle plot), and standard deviation (SD) of average catch (lower plot) over planning horizon. Numbers refer to rebuilding strategy.

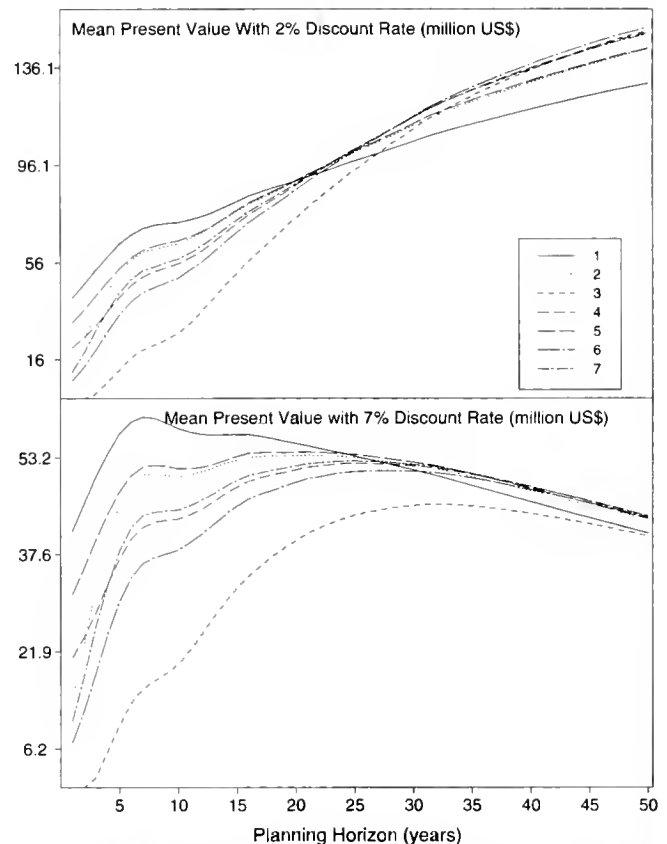


Figure 7. Mean present exvessel values with a 2% discount rate (upper plot) and a 7% discount rate (lower plot) for seven rebuilding strategies over planning horizon. Numbers refer to rebuilding strategy.

each of the six new strategies to outperform the status quo strategy (Table 4). For the equal tradeoff criterion, it took 36 y or longer. Performance based on the maximum present exvessel value criterion depended on the discount rate. At a 2% discount rate, it took 20–28 y for the six new strategies to outperform status quo (Table 4). At a 7% discount rate, performance was intermediate between maximum catch and equal tradeoff criteria, requiring ≥ 25 y. For these three criteria, Strategy 5 resulted in the shortest time and Strategy 3 took the longest time to outperform the status quo strategy. Overall, a long time was required for the other strategies to outperform the status quo strategy under these three criteria.

The best of the seven strategies was a function of evaluation criterion and planning horizon. Regardless of planning horizon, Strategy 3 had the highest probability of rebuilding and Strategy 4 resulted in the lowest probability of fishery closure (Table 5). Under maximum catch, equal tradeoff, and present exvessel value criteria, the status quo strategy was the best under a 10-y or less planning horizon (Table 5). Under the same criteria and a planning horizon of 30 y or longer, Strategies 5–7, which reduced harvest rate from the status quo level, were generally the best (Table 5).

Sensitivities to Natural Mortality, Handling Mortality, Measurement Error, and S-R Relationship

Results were very sensitive to natural mortality, handling mortality, and S-R relationship, but not to measurement error (Table 6). For a given rebuilding probability, all strategies took much longer to rebuild the population under a high natural mortality, a high handling mortality, and an autocorrelated S-R relationship than under a low natural mortality, no handling mortality, and a general S-R relationship. Under conditions of increased mortality and autocorrelated environmental variability, the conservative Strategies 3, 4, 6, and 7 had higher probabilities of rebuilding than the more liberal Strategies 1, 2, and 5.

With maximum catch, equal tradeoff, and present exvessel value criteria, it took a much shorter time for the six new strategies to outperform status quo under a low natural mortality, a 50% handling mortality rate, and a general S-R relationship than under a high natural mortality, no handling mortality, and an autocorrelated S-R relationship (Table 7). Generally, under these criteria, Strategy 5 outperformed status quo fastest, whereas Strategy 3 took the longest time to outperform status quo.

With a 10-y planning horizon or less, the status quo strategy outperformed the other strategies under maximum catch, equal tradeoff, and present exvessel value criteria for all parameter levels considered (Table 8). With a planning horizon of 30 y or longer, the status quo strategy was still the best for scenarios with no handling mortality and an autocorrelated S-R curve under the

maximum equal tradeoff and present exvessel value criteria. Under the maximum catch criterion and a long planning horizon (≥ 30 y), Strategy 6 outperformed the others for all scenarios, except those with an autocorrelated S-R curve and different handling mortality rates. Generally, Strategies 3–6 outperformed the other strategies under scenarios with high handling mortality and natural mortality over a long planning horizon (Table 8). A general S-R curve and low natural mortality, on the other hand, favored Strategies 2, 6, and 7 over a long planning horizon, whereas an autocorrelated S-R curve favored relatively low threshold strategies (Strategies 1, 4, and 5) (Table 8).

DISCUSSION

A desirable rebuilding strategy will represent a balance between short-term loss and long-term gain. The choice of strategy depends on management objectives or evaluation criteria and planning horizons (Hightower and Grossman 1987, Quinn et al. 1990). Our simulation results indicated that all seven strategies lead toward rebuilding of the depressed Bristol Bay RKC stock, although rebuilding rates differ. The status quo strategy performed best among the seven strategies with a short-term planning horizon ≤ 20 y under maximum catch, equal tradeoff, or present exvessel value criteria. For a 30-y planning horizon or longer, a 25% reduction in the status quo harvest rate produced the best results under maximum equal tradeoff and present exvessel value criteria with a 7% discount rate. Conversely, maximum catch and present exvessel value criteria with a 2% discount rate favored a strategy with a 50% reduction in the status quo harvest rate and an approximate 67% increase in the status quo threshold. Minimizing the probability of fishery closure required a 50% reduction in the status quo harvest rate but allowed retention of the status quo threshold level.

Our results indicate that rebuilding time is expected to be very long for Bristol Bay RKC, even under the most conservative harvest strategy. These crabs can live >20 y and take about 7 y to mature (Zheng et al. 1995a). The process of rebuilding the depressed RKC stock is slow because the mean generation time leads to a slow response in accumulating spawning stock to levels that enhance the chance for substantially improved recruitment. A long planning horizon is needed to offset short-term costs of reduced harvests during rebuilding. To protect a stock's reproductive potential and maintain a healthy population for the long term, a conservative strategy is needed to minimize the chance of population collapse. A crab population exploited with low harvest rates will survive unfavorable environmental conditions better than one heavily harvested (Zheng et al. 1997). Zheng et al. (1997) suggested reducing the status quo mature male harvest rate as a long-

TABLE 4.
Minimum years required to outperform the status quo strategy under a given criterion for Bristol Bay RKC.

Evaluation Criterion	Strategy					
	2	3	4	5	6	7
Maximum catch	21	28	22	21	23	22
Maximum equal tradeoff	40	>50	41	36	44	40
Maximum present exvessel value (2% discount rate)	21	28	22	20	23	21
Maximum present exvessel value (7% discount rate)	27	>50	28	25	31	28

TABLE 5.

The best of the seven strategies for a given combination of evaluation criterion and planning horizon for Bristol Bay RKC.

Evaluation Criterion	Planning Horizon (y)					
	5	10	20	30	40	50
Fastest rebuilding	3	3	3	3	3	3
Minimum fishery closure	4	4	4	4	4	4
Maximum catch	1	1	1	6	6	6
Maximum equal tradeoff	1	1	1	1	5	7
Maximum present exvessel value (2% discount rate)	1	1	5	6	6	6
Maximum present exvessel value (7% discount rate)	1	1	1	5	7	7

term robust harvest strategy. Our results showed that strategies with reduced harvest rates would outperform the status quo strategy if the planning horizon is >20 y.

Simulation results were sensitive to the S-R relationship, natural mortality, and handling mortality. The S-R relationship is one of the most important factors determining optimal rebuilding strategies (Hightower and Grossman 1987, Quinn et al. 1990, Ianelli and Heifetz 1995). The autocorrelated Ricker S-R curve emphasizes environmental effects on recruitment and thus favors rebuilding strategies with a low threshold level. Conversely, the general Ricker S-R curve has strong density-dependent effects on recruit-

ment and leads to conservative strategies to effectively rebuild the population. Although the benefits of rebuilding the population, in terms of catch and present value, were different under both S-R curves, the population was rebuilt much faster with a conservative strategy than with the status quo strategy. If recruitment is strongly autocorrelated, as suggested by the autocorrelated Ricker S-R curve, the extremely weak recruitment observed in recent years may continue in the near future. Under this scenario, probabilities of fishery closure for different harvest strategies may be much higher, and probabilities of rebuilding may be lower than our results showed.

TABLE 6.

Minimum years required to rebuild effective spawning biomass to 25,000 t under a given probability for Bristol Bay RKC with low and high levels of natural mortality, handling mortality, and measurement error, and general and autocorrelated Ricker S-R curves.

Rebuilding Probability (%)	Examined Parameter	Strategy						
		1	2	3	4	5	6	7
50	Low natural mortality	13	12	8	9	11	9	11
	High natural mortality	>50	>50	24	50	>50	36	>50
	No handling mortality	16	15	12	13	14	13	14
	50% handling mortality	>50	41	12	18	30	16	22
	Autocorrelated S-R curve	33	27	18	21	26	20	23
	General S-R curve	15	14	9	11	12	11	12
	No measurement error	24	20	12	14	18	14	15
	High measurement error	26	21	12	15	19	14	17
80	Low natural mortality	20	18	12	14	16	14	16
	High natural mortality	>50	>50	>50	>50	>50	>50	>50
	No handling mortality	29	25	20	22	25	22	23
	50% handling mortality	>50	>50	20	37	>50	29	42
	Autocorrelated S-R curve	>50	>50	32	38	47	36	41
	General S-R curve	26	22	12	16	19	15	18
	No measurement error	>50	35	20	27	34	25	29
	High measurement error	>50	41	20	28	37	27	32
95	Low natural mortality	28	24	16	18	22	18	21
	High natural mortality	>50	>50	>50	>50	>50	>50	>50
	No handling mortality	46	41	34	37	41	36	37
	50% handling mortality	>50	>50	34	>50	>50	47	>50
	Autocorrelated S-R curve	>50	>50	49	>50	>50	>50	>50
	General S-R curve	42	31	17	23	27	21	24
	No measurement error	>50	>50	34	43	>50	39	45
	High measurement error	>50	>50	34	47	>50	44	>50

TABLE 7.

Minimum years required for a rebuilding strategy to outperform the status quo strategy under a given evaluation criterion for Bristol Bay RKC with low and high levels of natural mortality, handling mortality, and measurement error, and general and autocorrelated Ricker S-R curves.

Evaluation Criterion	Examined Parameter	Strategy					
		2	3	4	5	6	7
Maximum catch	Low natural mortality	14	20	17	16	17	16
	High natural mortality	30	36	29	25	31	30
	No handling mortality	26	>50	34	29	36	30
	50% handling mortality	18	21	17	15	18	18
	Autocorrelated S-R curve	38	>50	37	31	43	38
	General S-R curve	16	21	19	18	19	18
	No measurement error	22	29	23	21	24	22
	High measurement error	21	27	21	21	22	21
Maximum equal tradeoff	Low natural mortality	29	>50	34	30	36	31
	High natural mortality	>50	>50	>50	27	>50	>50
	No handling mortality	>50	>50	>50	>50	>50	>50
	50% handling mortality	37	45	29	21	33	32
	Autocorrelated S-R curve	>50	>50	>50	>50	>50	>50
	General S-R curve	29	>50	33	29	34	30
	No measurement error	39	>50	41	36	44	40
	High measurement error	>50	>50	44	42	47	46
Maximum present exvessel value (7% discount rate)	Low natural mortality	17	33	20	18	20	19
	High natural mortality	48	>50	41	33	>50	48
	No handling mortality	>50	>50	>50	>50	>50	>50
	50% handling mortality	21	27	20	17	21	21
	Autocorrelated S-R curve	>50	>50	>50	>50	>50	>50
	General S-R curve	19	31	22	20	22	20
	No measurement error	28	>50	30	25	32	28
	High measurement error	27	45	27	24	28	27

We assumed a constant natural mortality in this study due to the relatively short planning horizon. Natural mortality for this population has been estimated as variable over crab size and time (Balsiger 1974, Zheng et al. 1995a, Zheng et al. 1995b). Zheng et al. (1997) allowed natural mortality to change over time to examine long-term robust harvest strategies. The constant natural mortality used in this study is close to the mean of natural mortalities used by Zheng et al. (1997) and NPFMC (1990). Low natural mortality would dramatically shorten rebuilding time, whereas high natural mortality would considerably reduce rebuilding probability, especially for a liberal harvest strategy. The reproductive potential of the population would be better protected with a conservative harvest strategy than with a liberal harvest strategy, given uncertainty in natural mortality.

Handling mortality is an important factor influencing optimal harvest strategies (Zheng et al. 1997). Handling mortality reduces future recruitment to fisheries by reducing both prerecruit abundance and spawning biomass. Besides mortality, handling may also produce sublethal effects on crabs, such as reduced growth (Kruse 1993). A high handling mortality of 50% favors a conservative strategy. The status quo strategy had little chance of stock rebuilding under a high handling mortality, whereas it performed well under the questionable assumption of no handling mortality.

Handling mortality for crabs was discussed in detail by Kruse (1993) and Zheng et al. (1997). Mortality rates for crab bycatch may depend on handling injury, air temperature, wind speed, shell

condition, and numerous other factors (Carls and O'Clair 1990, Kruse 1993, Murphy and Kruse 1995, Zhou and Shirley 1995, Zhou and Shirley 1996). The exposure of RKC to cold air reduces vigor, lowers growth, and leads to increased mortality during ecdysis in severe situations (Carls and O'Clair 1990). On the other hand, simulated deck and water impacts caused no increase in the mortality of RKC, although injuries to spines and rostrum increased with handling (Zhou and Shirley 1995, Zhou and Shirley 1996). Because not all potential contributing factors have been adequately studied, the level of handling mortality experienced in the Bristol Bay RKC fishery remains uncertain. Given uncertainties, we believe that handling mortality rates of 10–20% may be a reasonable assumption for purposes of our analysis. Careless handling practices or exposure to extremely cold air temperatures would probably result in mortality higher than this range, whereas careful handling and moderate air temperatures would probably result in mortality toward the lower end of the range.

To simulate bycatch, we assumed a 50% catchability for sublegal male and large female crabs in this study. However, the catchability in 1992 and 1993 increased to nearly 100% (Tracy 1994). Increased catchability can probably be attributed to the reduction in average soak time resulting from shorter fishing seasons and the institution of a 250-pot limit per boat. The increase of pot mesh size initiated in 1995 may result in a bycatch catchability closer to that observed before pot limitation. If the bycatch catchability is higher than 50%, handling mortality will be underestimated in our study. The combination of a 50% catchability, 40%

TABLE 8.

The best of the seven strategies for a given criterion and planning horizon for Bristol Bay RKC with low and high levels of natural mortality, handling mortality, and measurement error, and general and autocorrelated Ricker S-R curves.

Evaluation Criterion	Examined Parameter	Planning Horizon (y)					
		5	10	20	30	40	50
Maximum catch	Low natural mortality	1	1	6	6	6	6
	High natural mortality	1	1	1	5	6	6
	No handling mortality	1	1	1	2	2	7
	50% handling mortality	1	1	4	6	3	3
	Autocorrelated S-R curve	1	1	1	1	5	5
	General S-R curve	1	1	7	6	6	6
	No measurement error	1	1	1	6	6	6
	High measurement error	1	1	1	6	6	6
Maximum equal tradeoff	Low natural mortality	1	1	1	2	7	7
	High natural mortality	1	1	1	5	5	5
	No handling mortality	1	1	1	1	1	1
	50% handling mortality	1	1	1	5	4	6
	Autocorrelated S-R curve	1	1	1	1	1	1
	General S-R curve	1	1	1	2	7	7
	No measurement error	1	1	1	1	5	7
	High measurement error	1	1	1	1	1	4
Maximum present exvessel value (7% discount rate)	Low natural mortality	1	1	7	7	7	7
	High natural mortality	1	1	1	1	5	5
	No handling mortality	1	1	1	1	1	1
	50% handling mortality	1	1	5	4	6	6
	Autocorrelated S-R curve	1	1	1	1	1	1
	General S-R curve	1	1	2	7	6	6
	No measurement error	1	1	1	5	7	7
	High measurement error	1	1	1	4	4	4

legal male harvest rate (approximately corresponding to a 20% mature male harvest rate), and 20% handling mortality rate results in a 4% mortality rate for the population of sublegal male and large female crabs due to handling. Thus, a handling mortality rate of 20% or less does not have a large effect on the population as long as the bycatch catchability is 50% or lower and the mature male harvest rate is 15% or lower.

Two kinds of measurement error may occur during stock assessments: random and systematic (Rivard 1989, Zheng et al. 1993). We examined random measurement error in this study and found little effect on rebuilding strategies when assuming a standard deviation of 0.5. Zheng et al. (1993) showed that, for Alaska herring populations, optimal harvest rates are lower with high random measurement error than with low measurement error. Systematic measurement error results from consistently overestimating or underestimating population abundances. An increase in catchability would result in an overestimation of recent abundances by a catch-at-age analysis (Rivard 1989, Parma 1993, Hutchings and Myers 1994). Similarly, an incorrect assumption about the catchability of the trawl survey would cause systematic bias in abundance estimates by use of the "area-swept" method. Consistent overestimation of abundance will lead to overharvest and may lead to stock collapse if a liberal harvest strategy is adopted. A conservative harvest strategy helps protect against stock collapse if there is uncertainty about systematic measurement error during stock assessments.

Increased recruitment is the key to rebuilding the Bristol Bay RKC population. Yet, environmental factors that influence RKC

recruitment are beyond the control of fishery managers. We can, however, change fishing activities to promote rebuilding. Two kinds of fisheries affect crab abundances: nondirected fisheries (e.g., groundfish trawl, scallop dredge, and non-RKC pot) and the directed RKC pot fishery. In our study, we incorporated only the losses from the directed fishery and assumed that the bycatch in nondirected fisheries on RKC would be maintained at low levels. Data are unavailable to separately estimate unobserved mortalities from nondirected fisheries, so they have been incorporated into our estimates of natural mortality and S-R relationships. A combination of time-area closures and bycatch limits constrains bycatch in the groundfish fisheries to a relatively small percentage of the RKC stock. However, increases in bycatch and unobserved mortalities from nondirected fisheries could slow the recovery of Bristol Bay RKC during the rebuilding period.

RKC tend to have life history traits identified with K-selected species (Kruse 1993). Relatively low fecundity, slow growth, and delayed reproduction increase RKC vulnerability to recruitment overfishing, a condition that occurs when the spawning stock is reduced by fishing to levels incapable of ensuring an adequate production of young crabs. If the population collapses, it will take a long time to recover. After more than a decade of fishery closures, the Kodiak, Alaska Peninsula, Dutch Harbor, and Cook Inlet RKC populations have still not shown any signs of recovery from their crash in the early 1980s. RKC in Adak islands have remained at historically low levels since their decline in the mid-1970s, and recent small fisheries remain unproductive. Conversely, RKC in many bays of southeast Alaska have recently recovered from se-

vere population declines after the fishery was closed for a little over a decade. Of all of the RKC fisheries in Alaska, the small Norton Sound fishery has perhaps been the most stable, providing consistent harvest, during the past two decades. This population has been managed more conservatively than other RKC populations in Alaska; the harvest rate is one-half the rate applied to other Bering Sea RKC stocks, and the nearshore area has been closed to commercial fishing. However, even this stock is at risk because of recent uncertain harvest rates associated with the lack of a stock assessment survey since 1992.

Other crustacean species have had mixed responses to fishery closures or other rebuilding efforts. The Tanner crab (*Chionoecetes bairdi*) population in Prince William Sound and pink shrimp (*Pandalus borealis*) populations in the northern Gulf of Alaska have remained at very depressed levels after more than 10 y of reduced fishing effort, culminating in fishery closures. In contrast, Pribilof and St. Matthew Islands blue king crab (*Paralithodes platypus*) populations have gradually rebuilt through fishery closures or reduction of harvest rates, although the population abundances have yet to reach the record levels of the early 1980s. The squat lobster (*Pleuroncodes monodon*) population on the continental shelf of central Chile reached the highest observed biomass in 1991 after 3 y of fishery closure (Roa and Bahamonde 1993). As a final example, a refuge equal to 2% of the male snow crab (*Chionoecetes opilio*) fishing grounds was established off Kyoto Prefecture, Japan, in 1983; during the subsequent 5 y, catch rates from the fishing grounds surrounding the refuge were significantly higher than those of more distant fishing grounds (Yamasaki and Kuwahara 1990). The failed responses of some crustacean species to rebuilding efforts may be due to a change in environmental conditions. There is good evidence for decadal-scale shifts in physical and biological regimens in the Atlantic (e.g., Russell 1973) and Pacific Oceans (e.g., Beamish and Bouillon 1995) that

may retard stock rebuilding for some species during periods unfavorable to recruitment.

There is no guarantee that any management strategy will rebuild Bristol Bay RKC within a certain time horizon because of the unpredictability of environmental conditions. However, for planning horizons of >20 y, a strategy with a reduced harvest rate (or increased threshold) would enhance the chance of rebuilding Bristol Bay RKC and reduce the risk of further abundance decline under uncertain environmental conditions, natural mortality, and handling mortality.

On the basis of the results from this study and from the study on optimal long-term harvest strategies (Zheng et al. 1997), the Alaska Board of Fisheries, who establish fishery regulations in the state of Alaska, adopted a new harvest strategy for Bristol Bay RKC in March 1996. The new strategy expands the status quo threshold of 8.4 million mature female crabs to include an equivalent biomass threshold of 6,600 t of effective spawning biomass. It sets the mature male harvest rate to 10% when both the crab abundance is above threshold and the effective spawning biomass is below 25,000 t and to 15% when the effective spawning biomass is equal to or above 25,000 t. The maximum legal male harvest rate is reduced to 50%. The new strategy aims to achieve a balance between short-term gains in yield and fishing opportunity and long-term stability in yield and reproductive potential.

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APPENDIX. POPULATION MODEL

The following model was applied to male crabs. The female model is the same except that catch was replaced by handling mortality and molting probability was set equal to 1.0. The abundances by length and shell condition in any one year result from abundances the previous year minus fishing, handling, and natural mortality, plus recruitment and additions to or losses from each length class due to growth:

$$N_{l+1,t+1} = \sum_{l'=1}^{l+1} [P_{l',l+1} ((N_{l',t} + O_{l',t})e^{-M_{l',t}} - C_{l',t}e^{(1-M_{l',t})})m_{l'}] + R_{l+1,t+1};$$

$$O_{l+1,t+1} = ((N_{l+1,t} + O_{l+1,t})e^{-M_{l+1,t}} - C_{l+1,t}e^{(1-M_{l+1,t})}) (1 - m_{l+1,t}), \quad (A1)$$

where $N_{l,t}$ and $O_{l,t}$ are new- and old-shell crab abundances in length class l and year t , $M_{l,t}$ is instantaneous natural mortality in year t , $m_{l,t}$ is molting probability, $R_{l,t}$ is recruitment, y is lag in years between abundance assessment and the fishery, and $P_{l',l}$ is proportion of molting crabs growing from length l' to length l after one molt. $C_{l,t}$ is catch (legal males) or handling mortality (sublegal males and females). Recruitment is defined as recruitment to the model and survey gear rather than recruitment to the fishery. $P_{l',l}$, $C_{l,t}$, and $R_{l,t}$ are computed as follows.

Mean growth increment per molt is assumed to be a linear function of premolt length:

$$G_l = a + bv \quad (A2)$$

where a and b are constants. Growth increment per molt is assumed to follow a gamma distribution:

$$g(x|\alpha,\beta) = x^{\alpha-1} e^{-x/\beta} / (\beta^\alpha \Gamma(\alpha)), \quad (A3)$$

The expected proportion of molting individuals growing from length class l_1 to length class l_2 after one molt is equal to the sum of probabilities with length range (v_1, v_2) of the receiving length class l_2 at the beginning of the next year, i.e.,

$$P_{l_1,l_2} = \int_{v_1-v_2}^{v_2-v_1} g(x|\alpha,\beta) dx. \quad (A4)$$

where v is the midlength of length class l_1 .

For each year, effective spawning biomass, SP_t , was computed as

$$SP_t = r_t \sum_l [F_{l,t} WF_l], \quad v \geq 90 \text{ mm CL}, \quad (A5)$$

where $F_{l,t}$ is female abundance in length class l and year t , WF_l is mean weight of female crabs in length class l , v is the midlength of length class l , and r_t is the ratio of male reproductive potential, MRP_t , to total mature female abundance (≥ 90 mm CL), TMF_t , in year t , i.e.,

$$r_t = \begin{cases} MRP_t / TMF_t & \text{if } MRP_t < TMF_t \\ 1 & \text{if } MRP_t \geq TMF_t \end{cases} \quad (A6)$$

That is, if $r_t \geq 1$, then there are sufficient mature males to mate with all mature females, and so the number of spawning females is equal to the number of mature females. If $r_t < 1$, then not enough mature males are available to mate with all mature females, and the number of spawning females will be a fraction of mature females.

Male reproductive potential is defined as

$$MRP_t = \sum_l [(M_{l,t} + O_{l,t}) mm_l], \quad v \geq 120 \text{ mm CL}, \quad (A7)$$

where $N_{l,t}$ and $O_{l,t}$ are mature male crab abundances in length class l and year t with new- and old-shell conditions, respectively, and mm_l is the maximum number of females mated by a male in length class l (Table 1).

Annual effective spawning biomass, SP_t , was used to determine whether the population is above threshold, T . If $SP_t \leq T$, then no fishing is allowed; otherwise, the legal male harvest rate applied to legal crabs ($v \geq 135$ mm CL), NL_t , is

$$H_t = \min[E(NM_t/NL_t), MH], \quad (A8)$$

where E is mature male harvest rate applied to NM_p , mature male abundance (≥ 120 mm CL), and MH is the maximum allowable legal male harvest rate. Catch by length is

$$C_{l,t} = H_t (N_{l,t} + O_{l,t}), \quad \iota \geq 135 \text{ mm CL}, \quad (\text{A9})$$

and total yield, TC_p , is obtained by multiplying by the corresponding weight and summing over all lengths:

$$TC_t = \sum_l [C_{l,t} W_l], \quad \iota \geq 135 \text{ mm CL}. \quad (\text{A10})$$

Handling mortality is incorporated in the length-based model for female and sublegal male crabs. We assumed that catchability for large (≥ 90 mm CL) female and sublegal (95–134 mm CL) male crabs is 50% of that for legal male crabs (Zheng et al. 1997). Thus, female deaths due to handling are assumed to be

$$HD_{l,t} = 0.5 H_t F_{l,t} HM, \quad (\text{A11})$$

where HM is mortality rate for the bycatch. Deaths from handling for sublegal males are obtained by replacing female abundances with sublegal male abundances in Eq. (A11). Therefore, the magnitude of handling mortality is a function of harvest rate and mortality rate for the bycatch.

To account for the handling mortality of female crabs, effective spawning biomass is updated after fishing by modifying Eq. (A5) to deduct handling mortality from female abundance:

$$SP_t = r_t \sum_l [(F_{l,t} - HD_{l,t}) W F_l], \quad \iota \geq 90 \text{ mm CL}. \quad (\text{A12})$$

Handling mortality for sublegal male crabs is included in equations for male abundance by replacing catch, $C_{l,t}$, with deaths due to handling for $\iota = 95$ –134 mm.

Recruitment is separated into a time-dependent variable, R_t , and a length-dependent variable, U_l , representing the proportion of recruits belonging to each length class:

$$R_{l,t} = R_t U_l, \quad (\text{A13})$$

where U_l is described by a gamma distribution similar to that in Eqs. (A3) and (A4) with parameters α_r and β_r .

Annual recruitment is described by a general S-R model:

$$R_t = SP_{t-k}^{r1} e^{r2 - r3 SP_{t-k} + v_t}, \quad (\text{A14})$$

where k is recruitment age; $r1$, $r2$, and $r3$ are constants; and environmental noise $v_t = \delta_t + \alpha1 v_{t-1}$. δ_t was assumed as an $N(0, \sigma)$.



OPTIONS FOR HUMANELY IMMOBILIZING AND KILLING CRABS

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Australia

ABSTRACT Trials were conducted on the Australian giant crab *Pseudocarcinus gigas* (Lamarek) to evaluate methods to: paralyze by injection (so that no muscular response is observed); paralyze by bath; humanely kill for scientific purposes; and humanely kill for human consumption. Treatments tested were: freshwater bath, chilling, heating, prolonged exposure to air, hypercapnic seawater bath (carbon dioxide addition), 2-phenoxy ethanol bath, magnesium sulfate bath, benzocaine bath, MS 222 bath, chloroform bath, clove oil bath, AQUI-STM bath, xylazine-HCl by injection, and ketamine-HCl by injection.

Xylazine-HCl (16 or 22 mg/kg) and ketamine-HCl (0.025–0.1 mg/kg), administered by injection, appear to be the best techniques for paralyzing crabs for short periods. Where injection is impractical, crabs may be successfully paralyzed within 30 min by a bath treatment of clove oil (≥ 0.125 mL/L) or AQUI-STM (≥ 0.5 mL/L). Chloroform (1.25 mL/L; 1.5 h) and clove oil (≥ 0.125 mL/L; ≤ 60 min) baths appeared to kill crabs humanely and are useful options for scientific use; however, clove oil is preferred because chloroform poses a human health risk. Of the methods tested, only clove oil and AQUI-STM appear promising as treatments for the humane killing of crabs for human consumption.

KEY WORDS: Crabs, paralysis, humane killing, euthanasia, clove oil, anaesthesia

INTRODUCTION

Methods of paralyzing crabs can benefit many research situations involving live crabs: procedures may be conducted more efficiently, and trauma to the crab is reduced (Oswald 1977). Where the application is prolonged or the dosage is increased, humane killing may result, which is desirable for research and commercial uses of crabs. In commercial situations, it is important that quality is not harmed by effects such as autotomy, and toxic chemicals cannot be used. In Australia, recent changes to animal cruelty legislation have added another consideration to the commercial killing of crabs: that the crab be killed humanely.

Humane killing involves attempting to inflict as little pain as possible while killing the crab. Pain is a difficult, or perhaps impossible, aspect to measure in animals other than humans, so it is usually inferred from changes in behavior that seem to indicate distress (Chapman 1992, Cook 1996). These behavioral changes are not apparent when the muscular response is blocked by induced paralysis, so anesthesia (blockage of pain) is not assured, despite an apparent lack of distress. Likewise, the absence of behavioral indications of distress does not necessarily indicate that killing is painless. Nonetheless, in the absence of methods to quantitatively measure pain, techniques for killing or immobilizing animals where distress is apparently reduced are preferred to techniques that produce obvious distress. These techniques may also have additional benefits, such as easier handling of immobilised crabs in research.

Although numerous methods of temporarily paralyzing and killing crustaceans have been documented, many are slow or inconsistent and appear to cause trauma (Brown et al. 1996). This study reports the results of trials in temporarily paralyzing and killing the Australian giant crab *Pseudocarcinus gigas* (Lamarek) (Oziidae), a commercial species harvested across the temperate region of southern Australia below 34°S. A range of physical and chemical treatments was tested to establish which treatments were

effective and economical for this large species and also to note apparent trauma from treatments. Treatments were evaluated for the following applications: paralyzing by injection (appropriate for large crabs); paralyzing by bath (appropriate for small crabs); killing for research (toxic chemicals acceptable); and killing for commercial use (safe for consumption).

MATERIALS AND METHODS

Adult giant crabs (*P. gigas*) were collected from western Tasmania by commercial fishers and ranged from 1 to 7 kg, with most between 2.5 and 3.5 kg. Crabs were held in 4-m³ tanks with flow-through seawater and were only used if they exhibited normal avoidance of capture. Treatments were first tested in producing paralysis; crabs were then allowed to recover in tanks with flow-through seawater and were monitored for 2 days to assess any ill effects. Where the treatment was effective and did not appear to cause pain (see below), further trials were undertaken to establish appropriate dosages for producing temporary paralysis and to assess the treatment for humane killing. In some treatments, the crabs appeared to be severely harmed by the paralysis trial and recovery was not assessed.

Criteria for Assessing Pain, Paralysis, and Death

Although pain is impossible to quantify, changes in behaviors of experimental animals have been used to infer perception of pain (Chapman 1992, Cook 1996). In these trials, the treatment was considered to have caused pain when crabs dropped limbs (autotomy), tore at their appendages or abdomens, became tensed and rigid, or appeared to have muscle spasms.

Paralysis was considered complete when the abdomen could be easily lifted and chelae (claws) could not be used defensively. When recovery was to be assessed, crabs were removed from bath treatments before circulation of water over the gills ceased (externally observed by flow of water). The nervous systems of crabs have two centers—the cerebral and the posterior ganglions. Baker (1955) devised a simple system of testing if these centers were functioning, and thus if the crab was alive, by observing the response to stimuli applied to different appendages. Where no re-

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sponse was observed, the crab was classed dead. Baker's (1955) system was modified in this study to avoid the use of optical stimuli because giant crabs are deep sea animals and their spectral sensitivity may have been impaired by surface-level sunlight after capture (Cronin and Forward 1988). Consequently, the following tests were used to assess if crabs were dead:

- Antennal reaction: The crab does not retract the first antennae when the distal end is touched (cerebral ganglion).
- Maxilliped reaction: The third maxilliped (mouth frame) can be moved outward from the body and is not drawn back (posterior ganglion).

Treatment Strategy

Bath treatments were conducted in individual tanks of 20 L with continuous aeration (except hypercapnic seawater treatment). These tanks were filled with water from the larger holding tanks so that salinity (35 ppt) and temperature (range, 9–13°C) were not altered. Injections were made intravascularly through the coxal arthropodial membrane of a cheliped (Fig. 1). Doses by injection were made up to a maximum of 2 mL because volumes greater than this were considered difficult to administer. Physical methods tested for paralyzing were freshwater bath; chilling (5, 2, and -1.5°C); heating (17, 18, 20, and 24°C); and prolonged exposure to air.

Chemical methods tested as baths were hypercapnic seawater (CO₂ bubbled into bath through a graphite airstone); 2-phenoxy ethanol (maximum, 1 mL/L); magnesium sulfate (35 g/L); benzocaine (0.08 and 0.24 g/L, stock solution of 40 g/L benzocaine in acetone); MS 222 (tricaine methane sulfonate; 0.5 g/L); chloroform (1.25 and 2.5 mL/L in water and agitated); clove oil (0.015–1.0 mL/L, dissolved in ethanol); and AQUI-S™ (Fish Transport Systems,™ Petone, New Zealand)(0.015–1.0 mL/L). Chemical methods tested by intravascular injection were xylazine-HCl (0.6–22.0 mg/kg; as 2% solution, Rompun-Bayer™) and ketamine-HCl (0.01–0.05 mg/kg; as 10% solution, Ilium-Troy™).

Of these treatments, four were tried for humane killing: freshwater bath, chilling, chloroform, and clove oil. Chilling was achieved by the addition of ice slurry to 100-L tanks held in a refrigerated room. Heating was achieved by placing immersion heaters in 100-L tanks.

The number of crabs used for experiments varied (Table 1) because the response of individual crabs to some treatments was so

poor at very high doses that further trials were not warranted. Other trials were conducted opportunistically with industry, so large numbers were used, such as with prolonged exposure to air when 55 animals were monitored. The opportunistic nature of the trials prevented concurrent experimentation.

RESULTS

None of the physical methods appeared to be suitable for producing temporary paralysis; they were either ineffective, or they appeared to distress the crab (Table 1). There were also practical problems with the physical methods that rendered them unsuitable. Crabs were only affected by cold water temperatures close to freezing. Consequently, regular monitoring was required for the entire 2-h period needed to partially paralyze crabs, to ensure that the water did not freeze. Also, crabs revived when the temperature rose, so they recovered rapidly during experimental procedures.

None of the physical methods appeared suitable for humane killing (Table 2). Aside from ethical problems, prolonged exposure to air would take longer than 48 h and was not attempted. Heating appeared to cause distress to the crabs, and killing by a gradual increase in temperature was not attempted. Although a fresh water bath killed crabs, it did not appear to be a humane method. Chilling was ineffective.

Of the chemical bath treatments tested, only chloroform, clove oil, and AQUI-S™ produced what appeared to be relaxed temporary paralysis. Crabs treated with chloroform had poor recovery after paralysis and took longer to die compared with those treated with clove oil treatments. Chloroform solutions become saturated at approximately 6.17 mL/L at 10°C, which is considerably higher than the concentrations used in this study (1.25 and 2.5 mL/L). Consequently, the similar times for crabs to become paralyzed in the two concentrations of chloroform cannot be attributed to saturation of solution. The optimal concentration of clove oil for both paralyzing and killing was 0.125 mL/L because stronger doses did not produce faster effects (Fig. 2; Tables 1 and 2). The optimal concentration of AQUI-S™ for paralyzing was 0.5 mL/L (Fig. 2).

Both of the temporary paralysis treatments administered by injection were effective and acted rapidly. Unlike xylazine-HCl, where paralysis appeared to be painless, ketamine-HCl appeared to produce distress in the crabs; this was only momentary, however, because paralysis occurred within 45 sec.

DISCUSSION

Several of the treatments tested in producing paralysis were rejected because they were ineffective or because the dose required was too large for practical purposes: prolonged exposure to air, 2-phenoxy ethanol, magnesium sulfate, and MS 222. MS 222 is used widely in paralyzing finfish (Clark 1990) and was recommended by Ahmad (1969) for amphipods, although several other studies have confirmed that it is ineffective in decapods (Foley et al. 1966, Oswald 1977, Brown et al. 1996). MS 222 is believed to act at the nerve membrane affecting sodium conductance in finfish (Ryan 1992), and the ineffectiveness of MS 222 in decapods may be related to the absence of acetylcholine at these terminals (Oswald 1977). The large amount of magnesium sulfate required to paralyze large decapods was considered impractical in this study, and the same conclusion was drawn by Foley et al. (1966). For smaller animals, and thus smaller bath volumes, the technique may still have value (Gohar 1937).

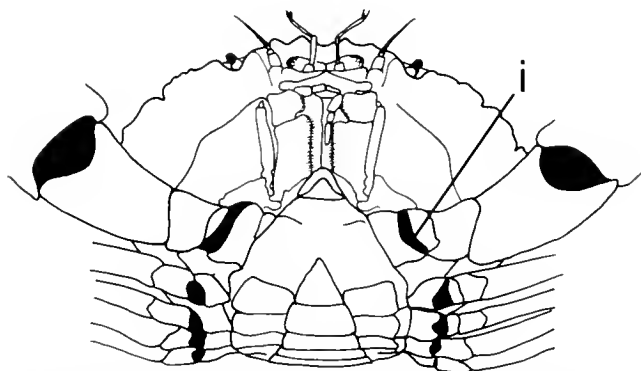


Figure 1. Ventral surface of a crab showing the site of intravascular injections (i). Treatments were introduced with the needle tip only slightly below the joint membrane to avoid penetrating muscle tissue.

TABLE 1.
Results of trials to assess the use of treatments for paralyzing.

Method	Time to Paralyze	Indication of Stress/Revival
Freshwater bath (n = 10)	Immediately became rigid and easily handled	Motionless and rigid for 10 min, then became very active. Autotomy occurred, and crabs tore at their abdomens and walking legs. No revival was attempted.
Chilling: 5, 2, and -1.5 C (n = 10, 10, and 60, respectively)	Unaffected after 14 h at 5 and 2 C. Mild paralysis in 2 h at -1.5 C (retained antennal, maxilliped, and limb movement).	Active at 5 and 2 C. Ice formed at -1.5 C, so the last segments (propodus and dactylus) of the limbs became frozen. All recovered within 45 min on return to 10 C and appeared healthy after 48 h. No effects of freezing were seen, although tissue damage is likely.
Heating: 17, 18, 20, and 24 C (n = 3 for all treatments)	Appeared unaffected at all temperatures tested, except 24 C. Mild paralysis at 24 C in 2 h.	Appeared uncomfortable and attempted to climb from the container as temperature rose. Although apparently paralyzed at 24 C, limbs constantly twitched. Recovery was rapid, and crabs appeared healthy after 48 h.
Prolonged exposure to air (n = 55).	No effect at 4 or 8 h. Less active after 14 h (8-12 C).	Crabs were vigorous after 14 h.
Hypercapnic seawater (n = 3)	Mean = 44 min (range, 33-60 min).	Appeared healthy after 48 h in seawater. Thrashed and crushed limbs. Although immobile, they were tensed and became rigid when returned to fresh seawater to recover. Some autotomy.
2-phenoxy ethanol: 1 mL/L (n = 1)	No effect after 14 h in saturated solution.	Slow recovery, incomplete after 48 h. No apparent effect.
MgSO ₄ : g/L in freshwater; 35 g/L in seawater (n = 6)	No effect at 4 h	Healthy 48 h after return to seawater. Active 48 h after return to seawater. Cost of chemicals made trials with higher doses unviable.
Benzocaine: 0.08 g/L (n = 1) and 0.24 g/L (n = 3)	2 h at 0.08 g/L; mean, 45 min and range, 20-55 min at 0.24 g/L	Apparent distress, tensed and rigid when immobilized. Autotomy occurred. Rapid recovery, crabs mobile within 10 min and healthy after 48 h.
MS 222: 0.5 g/L (n = 1)	No effect after 4 h.	One-tenth this dose (20 min) is used for killing finfish (Clark 1990). Trials at higher doses were unviable because of chemical costs.
Chloroform: 1.25 mL/L (n = 3) and 2.5 mL/L (n = 3)	60 min for all crabs	No apparent distress.
Clove oil: 0.015-1.0 mL/L (Fig. 2; n = 18).	Ineffective at 0.015 mL/L. Time at higher doses (≥ 0.03 mL/L) ranged from 85-16 min.	Slow recovery; crabs still sedated after 24 h, although apparently normal after 48 h. No apparent distress.
AQUI-S TM : 0.015-1.0 mL/L (Fig. 2; n = 14).	Ineffective at ≤ 0.06 mL/L. Effective at ≥ 0.125 mL/L in 20-70 min.	Rapid recovery (at 0.125 mL/L) and active 2.5 h after return to seawater. Appeared healthy after 48 h. No apparent distress.
Xylazine-HCl: 0.6, 1.2, 5.6, 11.2, 16, and 22 mg/kg. (n = 6)	Ineffective ≤ 11.2 mg/kg. Effective in 3-5 min at 16 and 22 mg/kg.	Rapid recovery and active 2.5 h after return to seawater. Appeared healthy after 48 h. No apparent distress.
Ketamine-HCl: 0.01, 0.025, 0.05, and 0.1 mg/kg. (n = 8)	Ineffective at 0.01 mg/kg; effective at 0.025, 0.05, and 0.1 mg/kg in 15-45 sec at all concentrations.	Rapid recovery: 25 min at 16 mg/kg, and 45 min at 22 mg/kg. Appeared healthy at 48 h. Cheliped became rigid immediately after injection; the other cheliped was thrashed, then relaxed.
		Recovery took 8, 15, 25, and 40 min at 0.01, 0.025, 0.05, and 0.1 mg/kg, respectively. Apparently healthy after 48 h.

TABLE 2. Results of trials to assess the use of treatments for humane killing.

Method	Time to Death	Comments
Freshwater bath (n = 10)	Mean, 4.6 h (range, 3–5 h)	Apparent distress (see Table 1).
Chilling: 2 and -1.5°C (n = 10 and 60, respectively).	2°C group alive at 24 h. -1.5°C alive at 6 h.	Appendage reactions occurred at -1.5°C, and limb movement was retained. Activity increased rapidly on warming.
Chloroform: 2.5 mL/L (n = 3)	1.5 h in all crabs.	No apparent distress.
Clove oil: 0.06–1.0 mL/L (Fig. 2; n = 17).	28–180 min, varying with concentration.	No apparent distress.

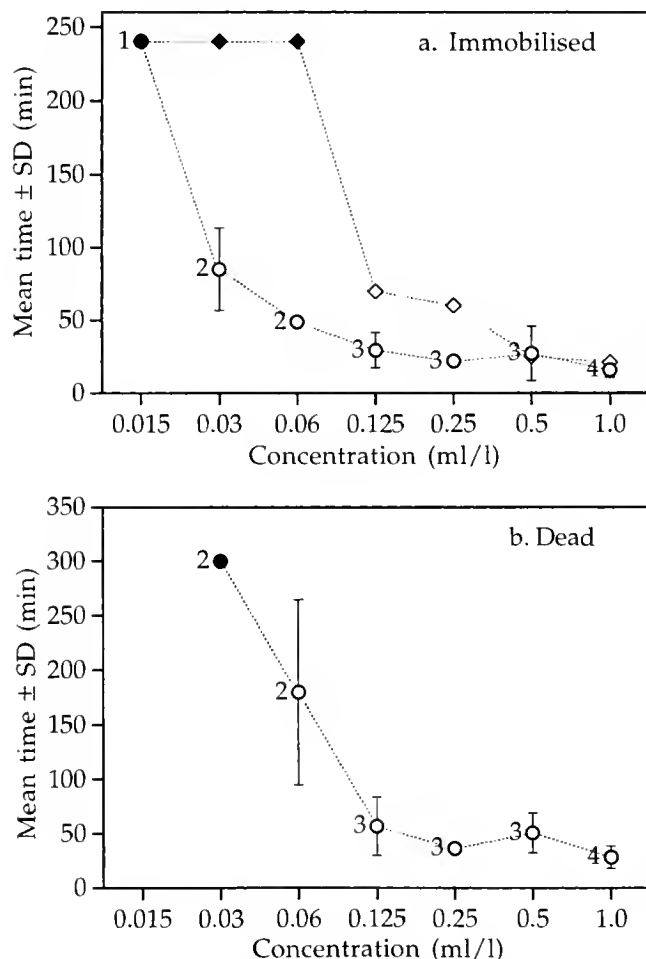


Figure 2. Effect of concentration of clove oil (circles) and AQUI-S™ (diamonds) on time taken to paralyze (upper) and to kill (lower) giant crabs *P. gigas*. Solid symbols represent trials terminated before paralysis or death occurred. Value labels next to means are numbers of crabs used. Tests with AQUI-S™ used only two crabs at each concentration, so no error values are presented.

Temporary Paralysis

Although many of the methods tested in this trial produced paralysis, some were only partially effective and others appeared to be unsuitable because of evidence of pain or distress during relaxation. Hypercapnic seawater has been recommended for paralyzing reptantian decapods (Smaldon and Lee 1979) and was also effective with *P. gigas*. However, the technique resulted in autotomy and thrashing of limbs in *P. gigas*, which indicates that the paralysis was not painless. Smaldon and Lee (1979) report that *Crangon* spp. and *Palaemon* spp. also exhibit distress when placed in hypercapnic seawater. Oswald (1977) assessed the use of benzocaine to temporarily paralyze *Cancer pagurus* L. and *Carcinus maenas* (L.) by injection and observed no effect. Benzocaine is widely used to produce paralysis in finfish research, where it is administered as a bath, as was done in this study with *P. gigas*. The bath solution of 0.24 g/L benzocaine produced paralysis in *P. gigas*, although there was some indication of pain, as with hypercapnic seawater.

None of the physical methods tested produced relaxed paralysis in *P. gigas*. A gradual increase in temperature was described as an effective and humane method of anesthetizing and killing large crustaceans by Gunter (1961) and was subsequently recommended by Smaldon and Lee (1979). This method was effective at paralyzing *P. gigas*, although animals showed signs of distress, contrary to the observations of Gunter (1961). Baker (1955) also tested the response of crabs to a gradual increase in temperature and concluded that the method was unacceptable on humanitarian grounds because indications of distress, such as autotomy, occurred unless the crab was already in poor health. After the publication of Gunter's (1961) conclusion on the use of gradual heating, objections were raised to the method on the basis that there was no evidence of an anesthetic effect (Baker 1962, Schmidt-Nielsen 1962).

Current Australian guidelines for the killing of crabs for scientific purposes recommend chilling as a humane method for paralyzing crabs, which can then be killed by sectioning to destroy ganglia (Reilly 1993). Although chilling may be useful for reducing activity in tropical or warmer water species, it was ineffective as a paralyzing technique for the temperate *P. gigas*. Freezing inevitably results in death in *P. gigas* but is of limited use in research because tissues are no longer suited for many applications, such as histology. Chilling has drawbacks that affect its use in all species—it is generally a slow and inconsistent technique (Brown et al. 1996), and it is ethically dubious because it involves subjecting the crab to conditions that it would normally avoid (Schmidt-Nielsen 1962).

Killing crabs by freshwater bath is one of the most widely used methods in Australia; it is termed "drowning" and is popularly considered a humane technique. Of all of the treatments tested for producing paralysis, "drowning" in a freshwater bath appeared to cause the greatest trauma because crabs dropped most limbs. Similar conclusions were drawn by Baker (1955) for *C. pagurus*.

Both xylazine-HCl and ketamine-HCl were particularly effective and produced paralysis in less than 5 min in *P. gigas*. Xylazine-HCl produces relaxation by central blockade of interneurons in the mammal (Oswald 1977), but the mode of action in crustaceans is unknown. Although injection of ketamine-HCl appeared to cause localized excitation, the apparent distress was only momentary because most crabs became paralyzed within 45 sec. Ke-

tamine-HCl is effective in paralyzing crayfish *Orconectes virilis* (Hagen), although the reported dose rate by intramuscular injection (90 mg/kg body weight; Brown et al. 1996) was considerably higher than that required by *P. gigas* by intravascular injection (0.025 mg/kg). The duration of paralysis in *P. gigas* treated with ketamine-HCl (8–40 min) also differed from that in *O. virilis* (>1 h; Brown et al. 1996). Dose rates of xylazine-HCl required to temporarily paralyze *P. gigas* (22 mg/kg) were less than reported values for *C. pagurus* and *C. maenas* (70 mg/kg; Oswald 1977), although the duration of paralysis was similar (around 45 min for all species). Two other chemicals, reportedly effective in other decapods, were not tested on *P. gigas* but warrant mention: procaine-HCl is reported to produce prolonged paralysis of 60 min in *C. pagurus* and *C. maenas* (Oswald 1977), and lidocaine-HCl is reported to produce shorter-duration paralysis of 20–25 min in *O. virilis* (Brown et al. 1996).

When the experimental animals are very small, injection is less practical than bath treatments; chloroform (>1.25 mL/L), clove oil (>0.125 mL/L), and AQUI-S™ (>0.5 mL/L) produced relaxed paralysis by this method. Chloroform has been used for many decades to kill decapods that subsequently remain relaxed for museum storage (Gohar 1937, Mahoney 1966), but the use of chloroform to produce temporary paralysis has been less well documented. Foley et al. (1966) attempted to paralyze *Homarus americanus* Milne Edwards, by chloroform bath, but concluded that too large a dose was required for practical purposes. The chloroform bath treatment was effective and inexpensive with *P. gigas*, although there are important limitations: the time to onset of paralysis (60 min) and recovery from paralysis (>24 h) was protracted, and chloroform poses a serious health risk to humans because of its hepatotoxicity. Clove oil was the superior bath treatment with respect to both time to onset of paralysis (as rapid as 16 min) and recovery (2.5 h). Clove oil is inexpensive and is likely to be effective over a wide range of species, given that it also produces paralysis in rabbitfish *Siganus lineatus* (Cuvier and Valenciennes) (Soto and Burhanuddin 1995). AQUI-S™ produced results similar to those of clove oil but may have limited application in paralyzing crabs for scientific purposes because higher doses were required. A potentially useful observation from the clove oil trials is that embryos of ovigerous giant crab females did not appear to be harmed by the treatment and continued through development to hatch.

Bath treatments of clove oil and AQUI-S™ may have commercial application to improve seafood quality and to reduce mortality during live transport. Reduction of stress during transport and before harvest is known to increase the quality of seafood (Lowe et al. 1993) and to decrease transport mortality (Paterson et al. 1994).

Humane Killing

Two of the treatments widely used in Australia for killing crabs were either ineffective or appeared to cause suffering: chilling and freshwater bath. Both chloroform and clove oil were effective, and

crabs did not appear distressed by the treatments. As discussed earlier, chloroform has long been used to kill crustaceans for museum collections (Gohar 1937, Mahoney 1966), where it is important that the animal does not autolyze limbs. Chloroform is hazardous to humans because of its hepatotoxicity, so it should only be used where all fumes can be removed.

Unlike chloroform, clove oil has the potential to be used for killing animals destined for human consumption, although the long-term chronic effects on humans are not yet known (Soto and Burhanuddin 1995). Cloves have been shown to delay the rancidity of seafood (Joseph et al. 1989), although the oil has a strong smell, which can alter the taste of the meat. AQUI-S™ is approved for use with food fish in New Zealand with zero withholding time; it produced paralysis in giant crabs, although higher doses were required than with clove oil. Unlike clove oil, AQUI-S™ does not have a strong odor, so is less likely to affect the taste of the meat. Further trials are warranted to assess the use of AQUI-S™ in the killing of crabs and to assess the effect on meat quality utilizing human sensory evaluation.

CONCLUSIONS

Several methods of paralyzing and killing crabs are clearly suitable for research situations. The two injectable treatments, xylazine-HCl (16 or 22 mg/kg) and ketamine-HCl (0.025–0.1 mg/kg), have much potential in research to reduce trauma to the crab, increase work efficiency, and reduce risk to humans from the chelae. Xylazine-HCl and ketamine-HCl act rapidly, so they can be readily applied in most research situations. Where injection is impractical, clove oil (≥ 0.125 mL/L) or AQUI-S™ (≥ 0.5 mL/L) baths were effective in paralyzing crabs although they both required around 20 min to act at optimal doses. A clove oil (≥ 0.125 mL/L) bath appeared to kill crabs humanely and is a useful option for research; crabs did not appear to experience trauma by this method, and there was no limb loss or other damage. Of the methods tested, only clove oil and AQUI-S™ appear promising as treatments for the humane killing of crabs for human consumption; however, both required long periods (≥ 25 min) to act, which may limit their commercial application. Baker (1995) described a method for killing crabs for human consumption by sticking, which involves piercing the nerve ganglia with an awl. This was not attempted with *P. gigas* because the sternum is exceptionally thick and difficult to pierce. However, in other species, sticking is likely to be a useful, and rapid, technique.

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DOMOIC ACID UPTAKE AND DEPURATION IN DUNGENESS CRAB (*CANCER MAGISTER* DANA 1852)

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ABSTRACT The potent marine neurotoxin domoic acid (DA) was detected in razor clams and Dungeness crabs on the Pacific Coast of the United States in 1991, resulting in temporary closures of these fisheries. Closures protect the health of human consumers of clams and crabs but impose significant economic losses to the communities that are dependent on these fisheries. Widespread closures, and in the case of the clams long-lasting ones, were necessary risk management strategies because our knowledge of DA uptake and movement through the food web is very limited. In order to resolve some of these issues and provide health managers with better information concerning this toxin, experiments were conducted on the accumulation and fate of DA in Dungeness crabs. Such information could provide enhanced safety, permit more efficient closures, and lessen the economic effect of future outbreaks.

In the first study, razor clams, containing known concentrations of DA, were fed to Dungeness crabs for 5 days to determine the uptake of the toxin by the crabs. Twenty-four hours after the crabs ingested an initial 960 µg of toxin, 260 µg of DA (27%) was found in the hepatopancreas (HP) of the crabs. At the end of 6 days, 68% (2,850 µg), from an accumulated 4,220 µg of ingested toxin, was present in the HP. DA was never found in the hemolymph or edible muscle of crabs in this experiment, but DA was found in the feces, indicating a route of depuration.

The second study examined the depuration of DA by crabs under fed and starved conditions. Crabs fed DA-contaminated clams for 4 days achieved an average concentration of 69.5 µg of DA/g of HP. After 7 days, crabs that were fed toxin-free clams three times per week showed a 38% reduction in DA concentration, to 43.4 µg of DA/g, whereas the average toxin concentration in the HP of crabs that were starved was reduced by only 4%, to 66.9 µg of DA/g.

In the last sampling, taken at 21 days, the concentration of DA in the HP of fed crabs decreased by 89% of the initial DA concentration to 7.6 µg of DA/g, but that of the starved crabs decreased by only 57%, to 29.7 µg of DA/g. Differences in mean concentrations between starved and fed crabs at 7, 14, and 21 days were significant. Additional measurements at 21 days showed the average weight of a starved crab's HP was only 53% of the fed crab's HP (25.7 vs. 48.7g). Although the mean weight of the starved crabs (770 g) was greater than that of the fed crabs (730 g), the difference was not significant.

KEY WORDS: Domoic acid, Dungeness crab, *Cancer magister*, razor clams, *Siliqua patula*, amnesic shellfish poisoning

INTRODUCTION

The Dungeness crab fishery of the Pacific Coast of the continental United States and Alaska is an important and well-established industry that supports a large domestic market as well as overseas markets. During the 1994 commercial season, 46 million pounds of Dungeness crabs (*Cancer magister*) were landed, with an exvessel value of over 63 million dollars (Anonymous 1995). There is also a large recreational fishery for Dungeness crab on the Pacific Coast that contributes to an important tourist industry. Between 1981 and 1991, an estimated 683,400 pounds of crab per year were caught recreationally in Washington alone (J. Odell, WA State Department of Fish & Wildlife, pers. comm. 1996).

In the fall of 1991, the Pacific Coast Dungeness crab fishery was involved in an outbreak of domoic acid (DA) poisoning when the toxin was found in the internal organs of the crab. The presence of high concentrations of the toxin in crab viscera resulted in a

temporary closure of the fishery. The closure had a significant economic effect on the industry and communities directly and indirectly connected to the harvest of crabs.

A potent neuroexcitatory amino acid, DA is a naturally occurring marine toxin that contaminated not only Dungeness crabs that year, but other marine species as well as seabirds. Although a diatom of the genus *Nitzschia* was suspected as the cause of the outbreak, it was never confirmed. Four marine phytoplankton species of the genus *Pseudo-nitzschia* [*P. multiseriata* (Hasle) Hasle, *P. delicatissima* (Cleve) Heiden, *P. australis* Frenguelli, and *P. seriata* (Cleve) Peragallo] are known producers of DA (Bates et al. 1989, Martin et al. 1990, Garrison et al. 1992, Work et al. 1993, Lundholm et al. 1994, Hasle 1995).

The consumption of DA-contaminated food by humans can cause mild to severe gastrointestinal illnesses and/or neurological symptoms such as disorientation and memory impairment. Although in high doses, DA can be fatal to anyone, the elderly and

health-compromised individuals are especially vulnerable. Because one of the symptoms of the poisoning can be varying degrees of loss of short-term memory (Perl et al. 1990a, Perl et al. 1990b, Zatorre 1990), and the vector was blue mussels, the DA intoxication was named amnesic shellfish poisoning. Stewart et al. (1990) considered DA intoxication symptoms more characteristic of dementia; nevertheless, it should be referred to by the more accurate name, DA poisoning (DAP), because fin fish are also a vector for the toxin.

To date, only two known outbreaks of DAP have been reported, both occurring on the North American continent. The first occurred in 1987 and resulted from human consumption of DA-contaminated, commercially cultivated, blue mussels (*Mytilus edulis* Linnaeus) from Prince Edward Island, Canada. These mussels were later shown to have fed on toxic phytoplankton, *P. multiseri* (Bates et al. 1989, Wright et al. 1989). Over 100 people suffered ill effects brought on by the toxin, and three deaths were attributed to DAP.

The second outbreak of DAP occurred in 1991 in Monterey Bay, CA, and involved brown pelicans (*Pelecanus occidentalis* Linnaeus) and Brandt's cormorants (*Phalacrocorax penicillatus* Brandt). These seabirds consumed anchovies (*Engraulis mordax* Girard) that had fed on DA-producing phytoplankton, *P. australis* (Fritz et al. 1992, Work et al. 1993). Shortly after the toxin's presence was noted in California, it was also detected in marine animals from the coastal waters of Washington and Oregon, where razor clams (*Siliqua patula* Dixon) and Dungeness crabs (*C. magister* Dana) were found contaminated with DA. Because of quick action by state and federal agencies and support from the Canadian scientists involved in the 1987 occurrence, no human seafood illnesses from DA were confirmed during this outbreak. As a result, the toxin's effect on the Pacific Coast of the United States was primarily economic, with the closures of the Dungeness crab and razor clam fisheries heavily affecting fishermen, processors, and local economies of the coastal communities dependent on those fisheries.

The initial testing of crabs for the presence of DA during the outbreak indicated that the toxin was confined to the viscera. Continued research has clearly demonstrated that DA is found only in the digestive system of Dungeness crab, primarily in the hepatopancreas (HP) (Wekell et al. 1994a, Lund 1995). Similarly, lobsters (*Homarus americanus*) are known to become toxic with paralytic shellfish poison (PSP), which like DA, contaminates the digestive gland (HP). The problem is significant enough that the Canadian government included lobster in its PSP-monitoring program (Watson-Wright et al. 1991, Lawrence et al. 1994). The presence of DA can pose a health risk to people who eat the HP or "crab butter." For public safety, the action level for the toxin in crab viscera was set at 30 µg of DA/g of viscera (U.S. Food and Drug Administration [FDA] 1993).

DA can also cause economic problems for the industry, especially with respect to crabs earmarked for whole cooked product markets. The presence of the toxin could require that crabs be butchered, cleaned, and cooked before being placed on the market, thus reducing profit margins or in some cases causing loss of market. More information about the fate of DA in Dungeness crabs, therefore, was needed not only by public health officials, but also by the industry to improve its ability to maintain a viable market.

The goal of this study was to provide information about the uptake and depuration of DA in Dungeness crabs to answer the

following questions: How rapid is DA uptake? Does ingested DA accumulate in crab tissues? Will crabs depurate accumulated DA? Do conditions of starvation or feeding affect the depuration of DA from crabs?

MATERIALS AND METHODS

Crabs

Two experiments were conducted. In the first experiment, Dungeness crabs were fed DA-contaminated clam meat to monitor the accumulation of DA in their HP. The second experiment monitored the depuration of DA from DA-contaminated crabs under starved and fed conditions.

Live Dungeness crabs used for these experiments were purchased from local commercial wholesalers. Fifty-two crabs were used in the first experiment, and 48 were used in the second experiment. Before their use in the experiments, each lot of crabs was randomly sampled and analyzed for DA to ensure that they were free of the toxin (<0.5 µg of DA/g of HP, as determined by high-performance liquid chromatographic [HPLC] analysis).

The weight of crabs in these experiments averaged 750 g (SD, ±84). They were marked with an identifying number and placed in live holding tanks that were supplied with gravel-filtered, flow-through seawater (~12 L/min). Dissolved oxygen levels in the seawater system ranged between 8.6 and 9.0 ppm, and water temperatures were between 12 and 13°C during the experiments.

Clams

Razor clams from the 1991 harvest, naturally contaminated with DA, were used to feed the crabs in these experiments. Purchased from a commercial source, the clams had been cleaned, canned, and frozen during the 1991 season, before the awareness of the DA outbreak and the discovery that DA concentrations in clams exceeded the FDA action level of 20 µg of DA/g of wet tissue (U.S. FDA 1993). The clams were subsequently removed from the market. Toxin levels in the clam tissues used in feeding the crabs ranged between 26.2 and 124.6 µg of DA/g. Commercially processed razor clam meats from Alaska, free of any detectable DA (<0.5 µg/g) were used for feeding crabs during acclimation to the laboratory environment and for the depuration study.

Feeding Crabs

Before being fed DA-contaminated clams, each lot of crabs was acclimated to its new environment for at least 24 h. In preparing the clams as feed, the product was first blotted with paper towels to remove excess thaw drip and then cut into 2- to 5-g pieces. Ten to 15 g of the clam pieces was weighed into individual trays, with each tray receiving the same weight of one or more clam parts (foot, body/mantle, or siphon).

A hand-feeding method, specifically developed for this work, isolated each crab in a net for individual feeding. Feeding was aided by the use of mechanical fingers, i.e., a spring-loaded extension tool (Lund 1995). Identification numbers on crabs made it possible to ensure that all crabs were fed once and only once at each feeding. Estimates of how much DA (µg) was consumed at each feeding were made by multiplying the weight of the clam meat portion fed to the crabs by the concentration of DA in a representative sample of the day's feed. The percentage of DA present in the HP 24 h postprandial was determined from the

product of the weight of the HP and its DA concentration, and dividing by the product of the weight of razor clam meat fed and its DA concentration, that is, DA-HP burden divided by DA-feed burden.

Sample Preparation and Storage

At each sampling, six to eight crabs were randomly selected from the holding tanks for dissection. All sample lots consisted of crabs taken 24 h postprandial, except for crabs sampled at 4 and 11 h in the uptake experiment. Before dissection, the crabs were drained, weighed, and pithed. The carapace was removed by cutting around its perimeter, and the epidermis covering the viscera was removed to expose the HP tissue. In the uptake experiment, all of the HP tissue was removed and weighed. In the depuration experiment, a subsample (ca. 10–22 g) consisting of HP material removed from the dorsal and ventral areas of the body cavity and lateral spaces under the carapace of each crab was composited and weighed.

To facilitate sampling procedures in experiment 1, some crabs were frozen whole at -10°C on arrival at the laboratory. When examined, the crabs were partially thawed to prevent excessive leaching, and the HP was sampled as described above and analyzed for DA. In Experiment 2, tissue samples were stored at 1°C until analyzed, usually within 24 h, or if necessary, frozen until analyzed. Drip was considered part of the sample and was mixed in before subsampling for analysis.

Analysis

DA concentration in the tissues was determined by the HPLC method of Quilliam et al. (1989, 1991), as modified by Hatfield et al. (1994), in which DA was extracted from HP tissues with an aqueous methanol solution and then purified and eluted through a strong anion solid-phase extraction cartridge with saline-acetonitrile solutions. Samples were run isocratically at 40°C with a reverse-phase C18 column at a flow rate of 0.300 mL/min. The mobile phase was water, acetonitrile, and trifluoroacetic acid (90/10/0.1, v/v/v). A photodiode array detector was set at 242 nm.

EXPERIMENTAL

Experiment 1—Uptake of DA

The purpose of this experiment was to determine the uptake, that is, the amount of toxin present in the HP after feeding Dungeness crabs DA-contaminated clam meat. After the first feeding of toxic clam meat (Time 0), the crabs were sampled at 4, 11, and 24 h to determine the time for the toxin to penetrate the HP. Thereafter, the crabs were fed every 24 h for a total of five feedings, i.e., 0, 24, 48, 72, and 96 h. Because sampling procedures followed each feeding by 24 h, the last crabs, fed at 96 h, were sampled at 120 h.

In this experiment, the DA burden of a crab was determined by multiplying the concentration of DA in sampled tissue by the total weight of the crab's HP material. The DA-HP burden is the total amount (μg) of toxin in the HP of a crab.

Experiment 2—Depuration of DA

The purpose of this experiment was to determine the effect of feeding on the depuration rate of DA from crabs. To get crabs to

an arbitrary toxin level over $50\text{ }\mu\text{g}$ of DA/g of HP, they were fed DA-contaminated clam meat each day for 4 days. Each crab was fed an average of 12.5 g/day of toxic clam meat for a total of 50 g containing $6,000\text{ }\mu\text{g}$ of DA. Twenty-four hours after the final toxic feeding, eight crabs were sacrificed and analyzed to determine the starting concentration of DA for the depuration experiment. At this time, the remaining crabs were evenly divided into the two treatment groups (fed and starved). Crabs in the fed group received from $5\text{--}7\text{ g}$ of DA toxin-free razor clam meat, three times per week for 3 wk. Random samplings of crabs from each group were taken at 7, 14, and 21 days, and HP samples were removed for DA analysis. In addition, the remaining HP tissues from all of the crabs sampled at Day 21 were removed, composited for each treatment group, and weighed and an estimated mean HP weight of a starved and a fed crab was determined.

Hemolymph samples from crabs in both treatment groups were obtained by severing a leg at the coxa and collecting the drip from live crabs at Day 1, i.e., 1 day after the last toxic feeding, and at Day 21. Fecal matter was gathered at the time of sacrifice, by extruding it from the distal portion of the hindgut. Fecal samples in both experiments were composited from three to eight crabs at each sampling and analyzed for DA as described above.

Statistics

Mean differences between treatments at 7, 14, and 21 days were tested by Student *t*-test using Statview (Abacus Concepts Inc., Berkeley, CA, 1992).

RESULTS

Experiment 1—Uptake of DA

This experiment monitored the uptake of DA in crabs fed toxic clam meat for five consecutive days. The crabs were initially fed clam meats containing $970\text{ }\mu\text{g}$ of DA (Time 0). When they were sampled postprandially at 4, 11, and 24 h, the crabs contained 155, 181, and $260\text{ }\mu\text{g}$ of DA, respectively, that is, 16, 19, and 27% of the DA that they were fed (Table 1). The average content of DA in the HP of the crabs continued to rise to 43% ($980\text{ }\mu\text{g}$) of the accumulative DA fed ($2,270\text{ }\mu\text{g}$) at 48 h and 56% ($2,160$ of $3,830\text{ }\mu\text{g}$) at 96 h. The last crabs sampled were fed $70 \pm 2.5\text{ g}$ of DA-contaminated clam meat in five feedings and achieved a mean DA-HP burden in the crabs of $2,850\text{ }\mu\text{g}$, or 68% of the total $4,220\text{ }\mu\text{g}$ of DA fed (Fig. 1).

Experiment 2—Depuration of DA

This experiment compared the simultaneous depuration of DA in two groups of Dungeness crabs. One group was fed DA toxin-free clams, and the other group of crabs was starved (Fig. 2). The crabs were fed DA-containing clam meat. After feeding for 4 days, a mean concentration of $69.5\text{ }\mu\text{g/g}$ of DA was achieved in the HP. At this point, the crabs were randomly distributed into two study groups: fed crabs were given razor clam meats containing no detectable levels of DA three times per week for 3 wk. After 7 days, the fed crabs showed a reduction in DA concentration of 38% ($69.5\text{--}43.4\text{ }\mu\text{g}$ of DA/g), whereas the starved crabs showed only a 4% decrease in DA concentration ($69.5\text{--}66.9\text{ }\mu\text{g}$ of DA/g). After 14 days, the DA concentration in the fed crabs decreased by 73%

TABLE 1.

Uptake of DA by Dungeness crab (*C. magister*) after periodic feedings with DA-contaminated razor clams (*S. patula*)

Feeding Times* (h)	Amount of DA Fed/Day per Crab (µg)	Cumulative DA (µg)	Sampling Times† (h)	No. of Crabs Sampled	DA in HP‡ (µg)	DA in HP as a % of DA Fed	Concentration of DA in HP§ (µg/g)
0 (initial)	970	970	0 (initial)	7	0	0	0
24	1,300	2,270	4	8	155	16	3.5 ± 0.6
48	1,070	3,340	11	7	181	19	4.7 ± 2.0
72	490	3,830	24	8	260	27	5.2 ± 1.5
96	390	4,220	48	6	980	43	18.4 ± 12.4
			72	0	NA	NA	NA
			96	8	2,160	56	32.6 ± 6.0
			120	8	2,850	68	57.5 ± 10.3

* Number of hours from first feeding of toxic clams. Crabs were fed once every 24 h for 96 h.

† Except for 4 and 11 h, crabs were sampled every 24 h postprandial.

‡ The amount of DA was calculated from the weight of each HP multiplied by its DA concentration.

§ Mean ± SD.

|| NA, not analyzed.

(69.5–19.0 µg of DA/g), but the DA concentration in the starved crabs decreased by only 28% (69.5–49.7 µg of DA/g). By the end of the experiment (21 days), both groups continued to depurate DA; however, the fed crabs showed the greatest decrease in DA concentration, with an 89% reduction (69.5–7.6 µg of DA/g), whereas the DA concentration in the starved crabs decreased by 57% (69.5–29.7 µg of DA/g) (Table 2). In each case, fed crabs had a significantly ($p < 0.02$) lower mean DA concentration than starved crabs when sampled at 7, 14, and 21 days.

On Day 21 of the second experiment, the mass of HP tissue in the starved crabs was noticeably less than that in the fed crabs. The mean whole weight of the HP from starved crabs, based on com-

posited material, was 56% of the mean whole weight of the HP from fed crabs (27.2g vs. 48.2 g). The average weight of a starved crab was more than the average weight of a fed crab (770 vs. 730 g) but was not significantly different.

A limited amount of data were collected to investigate a likely route of elimination of DA from Dungeness crabs. In the depuration experiment, DA was not detected (<0.5 µg/g) in samples of hemolymph obtained from the DA-contaminated crabs at Day 1, nor from the samples taken at Day 21. Further investigation of a route of toxin elimination involved collecting fecal material. After one feeding in the first experiment, the DA concentration in feces sampled after 24 h was 2.8 µg/g. After additional feedings, the DA content in fecal material was 2.8, 13.5, and 17.3 µg/g at 48, 96, and 120 h, respectively.

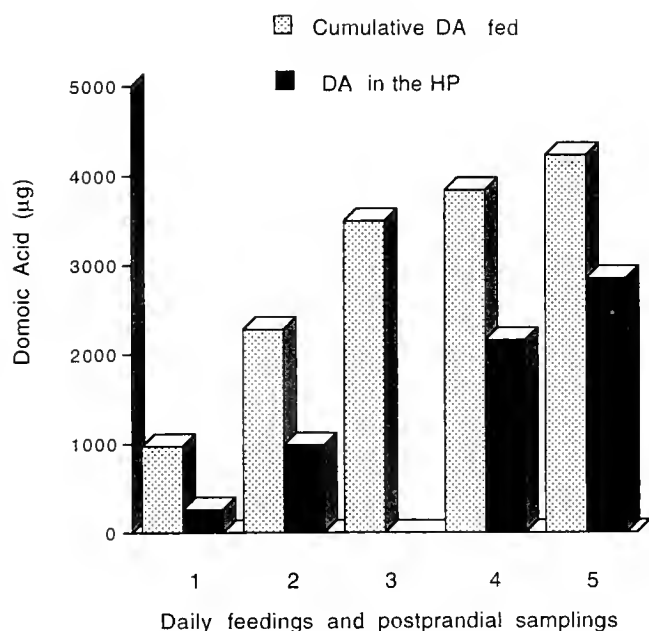


Figure 1. Summation of DA ingested by Dungeness crabs (*C. magister*) after daily feedings of toxic razor clams. Dark bars represent accumulated DA in the HP, expressed as HP burden. No samples were taken after the third feeding to maximize sample sizes after Feedings 4 and 5.

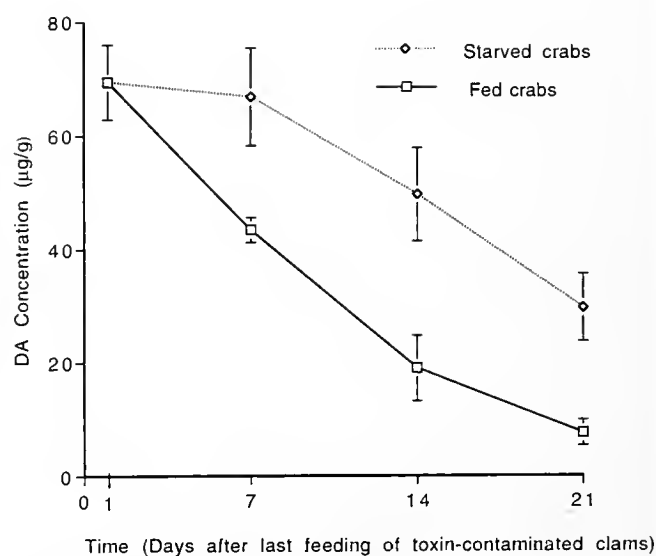


Figure 2. Effects of starvation and feeding on the concentration of DA in the HP of Dungeness crabs (*C. magister*) during depuration. Bars indicate SE, $n = 8, 6, 7$, and 7 for Days 1, 7, 14, and 21, respectively. Sampling at Day 1 represents the initial toxin level at the beginning of the experiment and occurred 24 h after the last toxic feeding.

TABLE 2.

Effects of starvation and feeding toxin-free clam meat on the depuration of DA by Dungeness crabs (*C. magister*).

Crabs	Time (days)*	n	Concentration of DA in HP (μg)†	SE‡	% of Initial Concentration
Fed	1	8	69.5 \pm 18.6	6.6	100
	7	6	43.4 \pm 6.0	2.2	62
	14	7	19.0 \pm 15.4	5.8	27
	21	7	7.6‡ \pm 6.2	2.3	11
Starved	1	8	69.5 \pm 18.6	6.6	100
	7	6	66.9 \pm 21.0	8.6	96
	14	7	49.7 \pm 21.8	8.2	71
	21	7	29.7# \pm 16.0	6.0	43

* Number of days from last feeding of toxic clams.

† Mean \pm SD.

||, #, # Means with the same symbols differ significantly as compared using the Student *t*-test ($p \leq 0.02$).

‡ Standard error.

Crabs were fed three times per week.

DISCUSSION

Feeding

Razor clams are a known source of food for crabs in the wild, when available (Tegelberg 1972, Stevens et al. 1982). Crabs in this study readily consumed the commercially processed, toxic razor clams, which suggests that razor clams can be a natural vector for the toxin as well as providing a laboratory method for introducing the toxin.

Crab behavior in this study appeared to be unaffected by the ingested toxin. It is not known if there is a limit to the amount of DA the HP of a crab can retain or if there is a point at which the toxicity would begin to affect the health of the animal. In the wild, Dungeness crabs have been found with concentrations as high as 252 μg of DA/g of HP (Chiang 1992).

Unequal feeding, which can occur from aggressive behavior in crabs and which is common with an ad libitum feeding method, was controlled by isolating the crabs individually in a net and using mechanical fingers to feed clam meat to each crab. The feeding technique yielded several other advantages over the ad libitum method. The hand-feeding method ensured that each crab ate only its portion of clam meat during a feeding and reduced the exposure time of meat to water, minimizing the possibility of leaching water-soluble DA from the clam meat. It also shortened the time period in which the crabs fed, so variation of digestion times between crabs was reduced. Furthermore, it identified crabs that were poor feeders, which were eliminated from the study.

Results reported by Wekell (1992), Wekell et al. (1994b), and Drum et al. (1993) showed that DA within the tissue of the razor clam is not uniformly distributed. For instance, the foot of the razor clam was often found to have much higher levels of DA than the siphon or body/mantle parts. Therefore, the feeding protocol described previously was adopted so that, theoretically, each crab received the same amount of DA at each feeding.

Sampling

Although the FDA action level uses μg of DA/g of viscera as a unit of measure, this study focused on the HP because it is the primary organ for digestion and because it is where the bulk of the

toxin was found. Clearly, the inclusion of the rest of the visceral contents plus entrained fluids and hemolymph would dilute any toxin present. This dilution would increase the possibility of error in the estimation of DA concentration in the crabs, for the purposes of this study. To simplify sampling of the HP, but to maintain representation, HP material was removed from three anatomical areas of the organ (dorsal, ventral, lateral) and composited for analysis. Twenty-four hours postprandial was chosen as the sampling time interval because that amount of time is considered to be normal for brachyuran crabs to completely digest and assimilate a meal (Icely and Nott 1992).

Occasionally, in this work, it was necessary to freeze samples to facilitate sample preparation and handling for chemical analysis. This technique was used without concern for loss of DA, because the toxin had been shown in the laboratory to be chemically stable to freezing, as an aqueous extract of homogenized razor clam meats (J. Wekell 1993, unpubl. data, Lund 1995) and in Dungeness crab viscera held in frozen storage for up to 1 y at -10°C (Quilliam et al. 1989).

A few studies have described the feeding of toxin-contaminated materials to crabs. One such study was a limited feeding experiment where Davies (1986) fed red rock crabs (*Cancer productus*) a single ad libitum feeding of clams contaminated with PSP and sampled the crabs over a 24-h period. At 4 h, there was a low concentration of toxin in the viscera, but no toxin detectable in the muscle tissue. After 24 h, high levels of the toxin were found in the viscera, little or no toxin was found in the stomachs, and none was detected in the muscle tissues. Davies concluded that PSP toxins accumulate in the viscera, but not in the muscle tissue of the crabs, a finding similar to the results of this study.

In previous work, Foxell et al. (1979) described feeding PSP-toxic clams to a group of rock crabs (*Cancer irroratus*) for 15 wk. After dividing the crabs into two lots, one lot was starved for a week and the other was fed nontoxic clams. Analyses showed that after 1 wk, the fed crabs had 138 μg of PSP/100 g of HP and the starved crabs had 242 μg of PSP/100 g of HP. It is also possible that the higher concentration in the starved crabs was partly due to loss of HP mass rather than entirely from retention of the toxin. Our data also showed that feeding increased the depuration process in crabs, suggesting that depuration in Dungeness crabs may be related to metabolic processes.

In a publication by Shumway (1990), it was noted that "toxins in the gastrointestinal tract (e.g., *Mytilus*) are eliminated more readily than toxins bound in tissues (e.g., *Placopecten*, *Spisula*, and *Saxidomus*)."

In DA-depuration studies by Novacek et al. (1991, 1992), blue mussels (*M. edulis*) showed a high rate of depuration and the toxin appeared to be retained primarily in the gut lumen, as previously reported by Wright et al. (1989). Mussels with an initial concentration of 50 μg of DA/g held under controlled laboratory conditions had only residual amounts of DA remaining after 72 h. Novacek et al. (1992) also studied the conditions of starvation and feeding on blue mussels and found that the fed mussels consistently depurated DA more rapidly than the starved mussels. However, the differences were not considered significant. Size of animal and environmental conditions, such as water temperature, were also shown to influence the rate of depuration.

In contrast to blue mussels, razor clams distribute DA throughout their various body tissues (Wekell et al. 1992, Drum et al. 1993, Wekell et al. 1994a). Depuration studies conducted by Drum

et al. (1993) and Horner et al. (1993) found that razor clams showed little or no loss of DA after 3 mo.

Earlier work in this laboratory showed that DA accumulated only in the HP of Dungeness crab and was not found in the edible meats of body or legs (Wekell et al. 1994a, Lund 1995). Because DA did not enter the edible muscle tissue of live Dungeness crab, but was found in the HP and feces, it was reasonable to assume that depuration would occur.

In conclusion, the results of this study showed that Dungeness crabs absorb DA quickly and may eliminate some of the ingested toxin quickly as a part of the digestive process. Also, toxin accumulated in the HP with daily feedings of DA-contaminated clams and effectively depurated from the HP over a 3-wk period once the toxic feedings ceased. The depuration proceeded at a faster rate when crabs were fed than when they were starved. DA was found

in the feces of both starved and fed crabs during the experiments, which confirmed one route of toxin elimination. DA was not found in the hemolymph. Because Dungeness crabs filter hemolymph through the antennal glands, which process urine (Icely and Nott 1992), urine was considered an unlikely route of toxin elimination. It was also noted that starvation caused a loss of weight in the HP mass during 3 wk of depuration, which could result in discrepancies when interpreting toxicity levels.

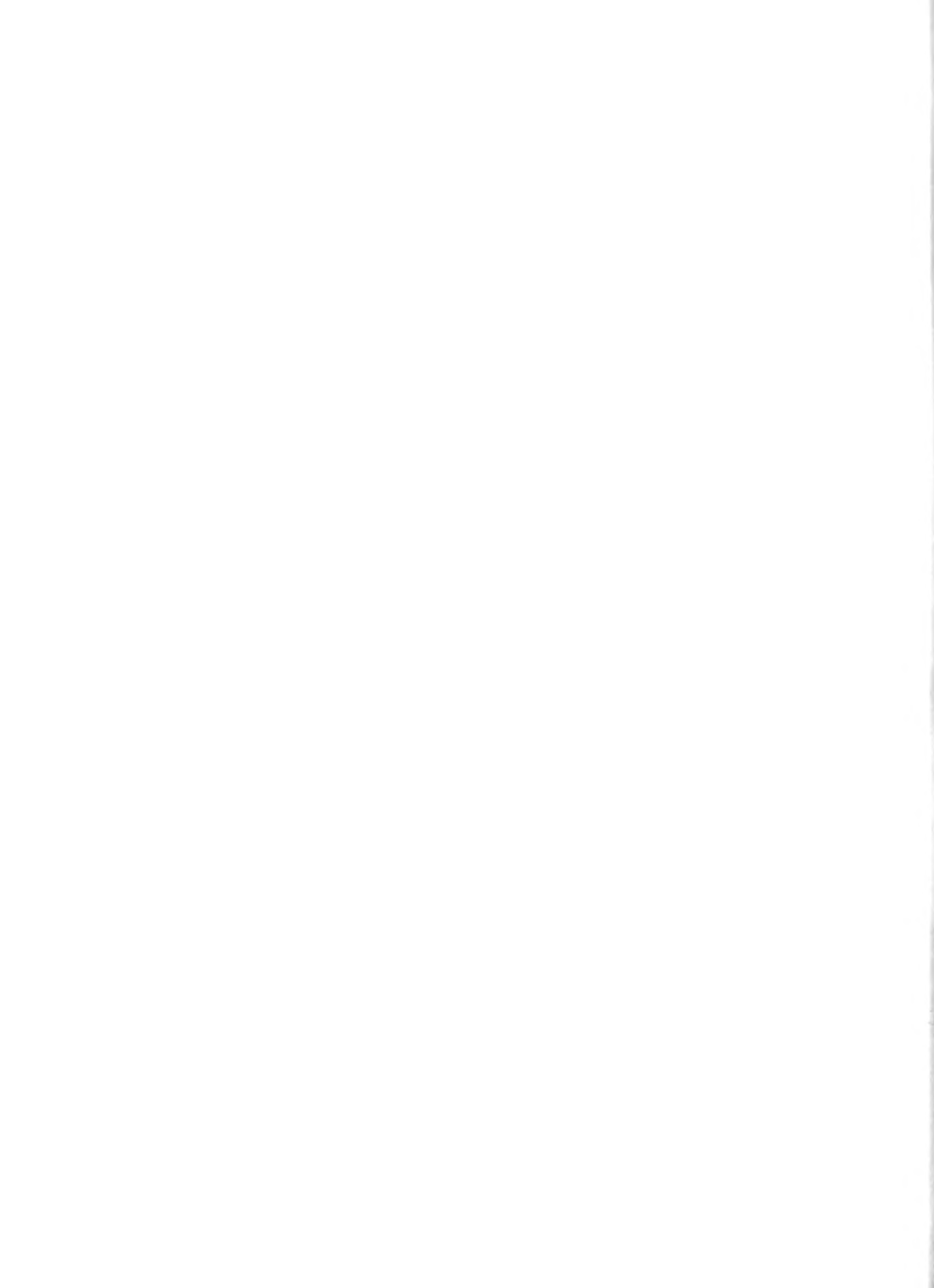
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ULTRASTRUCTURE OF THE CYST SHELL AND UNDERLYING MEMBRANES OF THE BRINE SHRIMP *ARTEMIA FRANCISCANA* KELLOGG (ANOSTRACA) DURING POSTENCYSTIC DEVELOPMENT, EMERGENCE, AND HATCHING

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ABSTRACT Cyst components and their products (shell, cuticles, membranes, embryo, prenauplius, nauplius 1) were examined with electron microscopy and identified in all stages of postencystic development through to the emergence of the prenauplius and the hatching of the nauplius 1. At prenauplius emergence, the shell (tertiary envelope) cracks open in a straight, smooth, 180° arc, while the first embryonic cuticle (EC1) separates in a jagged fashion, along a fracture seam between distinct polygonal plates. These plates differentiate within fibrous lamellae lying in concentric spheres between the outer and the inner cuticular membranes of the EC1. The second embryonic cuticle (EC2) forms after cyst uptake of water and initially adheres to the inner cuticular membrane (ICM) of the EC1. If hatching is interpreted as when the nauplius 1 is free from the EC1 and EC2 and able to swim, then we identify three methods of emergence but only one of hatching, as follows. From its shell, (1) the prenauplius emerges in a tapered EC2 bag, caudally attached to (or free of) the ICM, and then escapes from the EC2 as a swimming nauplius 1 (the only method of hatching); (2) the nauplius emerges halfway from the shell but without the ICM or EC2 over its head (it cannot escape from the shell and dies); or (3) the prenauplius emerges in an oval bag composed of the ICM and the EC2 (and the nauplius 1 differentiates within, but fails to escape and dies). During postencystic development and before the formation of the EC2, the embryo first secretes a fine, granular, extracellular matrix next to the ICM, and then between folds of the expanding cellular surface of the developing embryo. Next, the embryo forms the EC2, and the extracellular matrix is now between the ICM and the EC2, becoming exposed to the hatching medium only when the ICM breaks. During late embryogenesis, the EC2 forms over differentiating paired anterior appendages (and elsewhere), extending its surface area beyond that still adhered to the ICM. Finally, before hatching occurs, the EC2 inflates during prenauplius emergence, peeling off the surface on which it was formed; soon, the EC3 exoskeleton becomes continuous on its surface and mature, thus completing differentiation, and the nauplius 1 escapes the EC2 and becomes free swimming.

KEY WORDS: Hatching, cuticle, membranes, emergence, embryo, nauplius

INTRODUCTION

At the time of their release into the water, from late summer through late fall, the diapause cysts of *Artemia franciscana*, Great Salt Lake biotype (Utah), become hemispherical (unless hydrated by rain or melted snow) and are about 0.22 mm in diameter. Although such cysts are developmentally arrested, they are nonetheless metabolically highly active when first released (Clegg et al. 1996). Each cyst contains a single embryo, and thus this structure is analogous to a seed of flowering plants. If cysts are released by females into hypersaline water, they become dehydrated, as evidenced by their collapse inwardly on one side, becoming cup shaped and metabolically inactive, like seeds. Washed up on the shoreline in the fall and winter months, these cup-shaped cysts dry out and are about 0.21 mm in their widest diameter along their rim (Rosowski, unpublished; $n = 100$). On reentering the lake after spring rains, cysts swell, but unlike seeds they swell only on their collapsed side, becoming 0.22-mm spheres. That is, they increase "in volume but not in diameter" (Myint 1956). Viable cysts contain 4,000-cell embryos (Nakanishi et al. 1962) surrounded by a sturdy pigmented shell (tertiary envelope) that protects them from potential shoreline damage by abrasion and sunlight. Cysts can be dried and rewetted repeatedly, and their embryos will retain viability through many such cycles (Morris 1971). The cyst, with its shell and membranous coverings over the embryo, must have special adaptive features to allow for the physical changes accompanying repeated hydration and dehydration cycles that might occur and adversely influence postencystic embryonic development. De-

tailed structural studies are an important prerequisite to the accurate interpretation of the physiological and mechanical properties of the shell and of the cuticles and membranes associated with brine shrimp postencystic development, emergence, and hatching. Furthermore, there is a need to integrate the interpretation of cuticles and membranes described with light microscopy (Myint 1956, Sato 1967a, Sato 1967b, Belk 1987) with those observed with electron microscopy. In the words of Freeman (1989) "Clearly, further research on these interesting and complex membranes must be done before the role of each in development can be understood."

This study incorporates observations from scanning electron microscopy of critical-point dried (CPD), fractured whole cysts and shells from hatched cysts. In addition, we used scanning and transmission electron microscopy (SEM and TEM) to study shelled cysts in postencystic development to emergence and hatching. To follow the discussion, a definition of terms is in order. The commercially processed cysts of brine shrimp are referred to as shelled cysts when they have retained their tertiary envelope, or shell-free or deshelled cysts when the tertiary envelope has been treated with a deshelling solution until the embryos become orange (decapsulation). The shell consists of: an outer surface lamella, a cortical region, an alveolar region composed of three subregions, and a tertiary envelope base (Lee et al. 1994). Next to the shell is the first embryonic cuticle (EC1), which consists of a complex outer cuticular membrane (OCM) followed by a fibrous, lamellar region delineated as polygonal plates adjacent to a middle area often without lamellations, and a distinctive inner cuticular mem-

brane (ICM) that typically separates from the rest of the EC1 during prenauplius emergence. When cysts with shells or deshelled cysts are incubated in previously aerated saltwater, the postcystic embryo produces the second embryonic cuticle (EC2, or hatching cuticle) and, finally, a nauplius 1 exoskeleton outside the plasma membrane of its epidermal cells, the third embryonic cuticle (EC3, *sensu* Belk 1987). The embryo at the time of emergence, but without a continuous third embryonic cuticular surface (EC3 immature) and before it is free swimming, is referred to as a prenauplius. The nauplius 1 is defined as having an unbroken EC3 surface (mature), but it would not necessarily be free swimming. The general term nauplius is used when it is unclear as to whether the emerged stage is a prenauplius or nauplius 1. Two modes of complete emergence are described here, and one incomplete, with the nauplius surrounded by one or two membranes, which appear as a bag, or none at the head. Once free of the bag, the nauplius 1 is considered hatched (first swimming stage). The ultrastructural details presented here extend, integrate, and assist in the interpretation of the light (Myint 1956; Sato 1967a, Sato 1967b) and electron microscopy reports by Lee et al. (1994) and Rosowski et al. (1995) and the previous ultrastructural work of Morris and Afzelius (1967), Anderson et al. (1970), Wheeler et al. (1979), Trotman et al. (1987), and Trotman (1991).

MATERIALS AND METHODS

Utah biotype cysts, *A. franciscana* Kellogg, from Sanders Brine Shrimp Company L.C. (Ogden, UT), were imbibed and hatched over 24 h in previously aerated saltwater (NaCl 29 g/L + NaHCO₃ 6 g/L; or in Instant OceanTM, Aquarium Systems, Inc.) with just NaCl 35 g/L; to induce the emergence of oval-bagged prenauplii, as described by Sato (1967a, 1967b). Whole, water-imbibed cysts in early postcystic development were cut with a single-edged razor blade to expose their interior. Hydrated cysts that had cracked open and were in the process of hatching were fixed overnight in 3% glutaraldehyde in saltwater (Instant OceanTM), washed in saltwater, postfixed in 1% osmium tetroxide in saltwater, washed in saltwater, dehydrated in an ethanol series (25, 50, 75, 95, and 100% four times), and embedded in Epon 812 for TEM or CPD with liquid carbon dioxide in a Sorval critical-point dryer for SEM. The CPD cyst shells and unhatched cysts were fractured with the sharp edge of a single-edged razor blade before sputter-coating with gold/palladium (Denton Sputter Coater Desk II) for SEM. To study cysts in the process of shell dissolution and the surface of whole postcystic embryos, a standard deshelling method was used (Aqua fauna BioMarine, Hawthorne, CA) with whole, dried cysts. SEM samples were studied on a Cambridge Instruments S-90 stereoscan and photographed with Polaroid 55P/N film. Shell-free nauplii 1 that had emerged in oval bags (ICM + EC2) were fixed whole, as were cysts chemically treated with a deshelling solution. Thin sections for TEM were cut on a LKB Ultratome III and stained with uranyl acetate and lead citrate. These samples were then observed and photographed on a Philips 201 TEM operated at 60 kV.

RESULTS

The Shelled Cyst Hatching: SEM Images

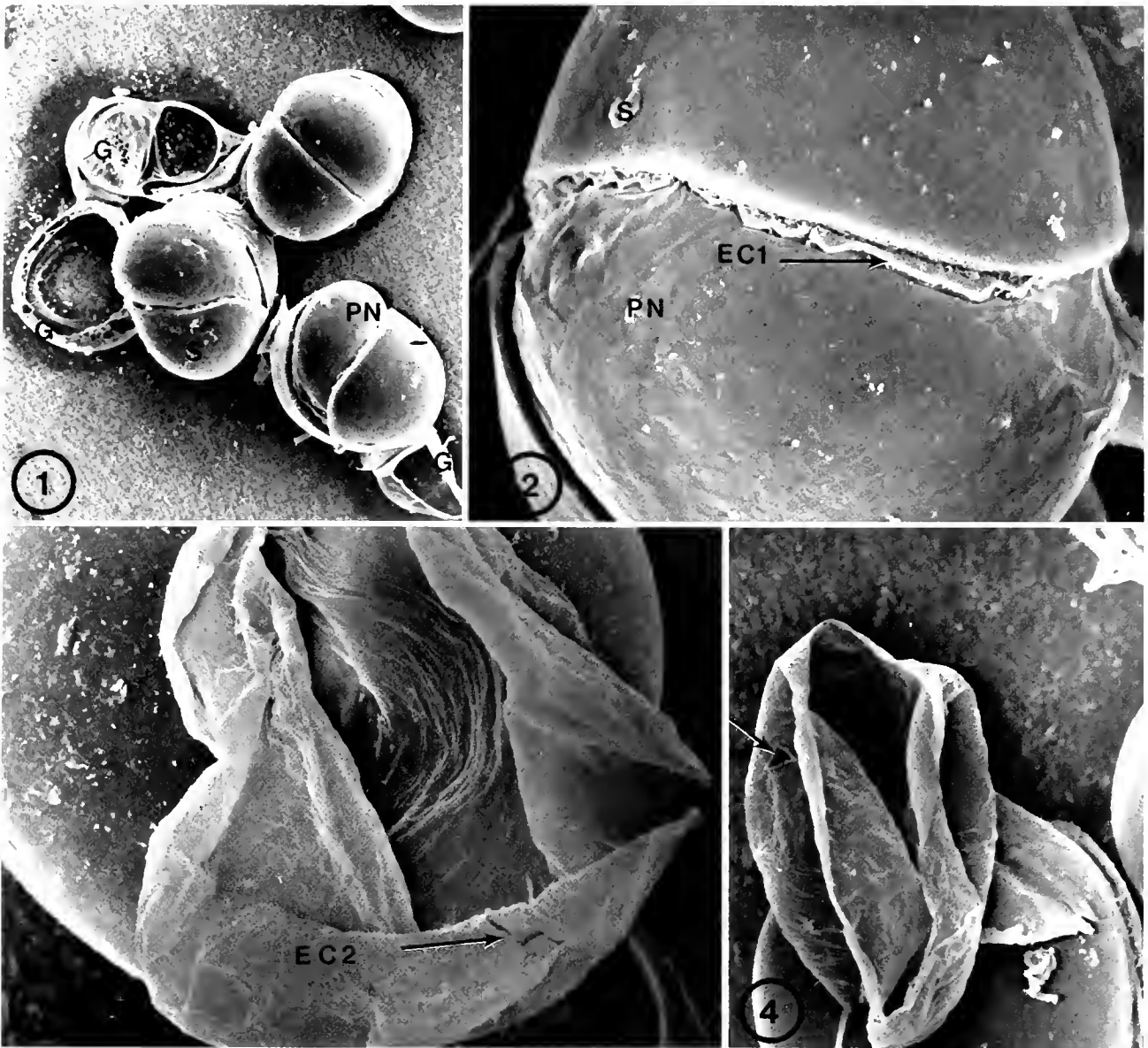
SEM of brine shrimp cysts in the process of hatching revealed six distinct regions within the shell, as previously reported (Lee et al. 1994). In addition, there were several membrane configurations

with respect to what remained in the shell or went out with the prenauplius on emergence from the shell. Although some of these configurations have been previously reported, they have been inadequately documented or are unreported from an ultrastructural viewpoint.

When prenauplii emerge, they may do so in synchrony, and with a membranous covering, before they fully escape from the shell (E1 stage; Fig. 1, and cf. Fig. 28). Whether or not this covering is a single or double membrane could not be determined with confidence in surface view with SEM (Figs. 1 and 2). An examination of a hatched cyst shell in a cracked area most often revealed a jagged edge, with a smooth edge superimposed over it (Fig. 2). The smooth edge was from the tertiary envelope (shell), whereas the jagged edge was along the polygonal plates of the first embryonic cuticle, a cuticle formed within the female (but by the embryo) before release of the cyst (EC1, *sensu* Belk 1987). In the typical emergence mode, the prenauplius emerges from the cyst shell enveloped in a tapered bag (parachute or umbrella of others). If cysts are attached to a substrate with an adhesive, then the head region of the parachute is upward (bag tapers downward), but if cysts are free floating, they may end up in the surface microlayer of the hatch medium and the parachute hangs downward from the floating shell to which it is attached.

The nauplius 1 eventually leaves two membranes behind, distinct in their surface morphology once discarded (Figs. 3–7). The most prominent, the one that surrounds the fully emerged prenauplius, is the second embryonic cuticle (EC2, the "hatching membrane" or cuticle), which was always wrinkled (Figs. 3–5). The other membrane to which the EC2 is typically attached caudally is the ICM of the EC1, and it is without wrinkles and featureless (Fig. 5). The EC2 is usually carried out of the cyst shell by the prenauplius, which it surrounds. Sometimes it was so closely associated with the EC1 that it tore in a jagged fashion (Fig. 3), or occasionally, what may be a piece of the shell remained attached to it (Fig. 4).

In the course of our comparisons of SEM and TEM images of similar stages of development, we identified two methods of complete emergence from the cyst shell and one incomplete. In the first and most common, the prenauplius emerged taking the entire EC2 with it. This created the well-known tapered bag or parachute attached at the caudal end to the inner surface of the ICM (Fig. 5, left) which in turn was attached to the shell. The nauplius 1 then escapes from the EC2 (hatches) (Fig. 5, left). Alternatively, the nauplius in the parachute moves away from the shell, leaving the ICM behind but pulled away from the shell surface and exposed in the shell crack (Fig. 5, right cyst). Eventually, the nauplius 1 (first larva, the L1) escapes from its membranous coverings with its lateral appendages usually beating at this point, but still closely oppressed (Fig. 8). The nauplius 1 grows into a metanauplius 1 (L2) within 24 h (Fig. 9). In the second method of emergence, the nauplius emerged only halfway out of the shell and was naked at the head, that is, with its exoskeleton exposed and immature (the EC3, Fig. 7). The ICM and EC2 remained largely within the shell, with broken edges evident around the middle of the nauplius (Fig. 7). However, we could find no evidence that this partially emerged nauplius was ever able to escape from the shell and become free swimming (hatch). In the third method of escape from the shell, examples of which were produced in significant numbers by hatching cysts in a medium containing only NaCl (i.e., without NaHCO₃), the prenauplius emerged in a firm, untapered oval bag (Fig. 6) that was without widespread surface wrinkles (when ex-



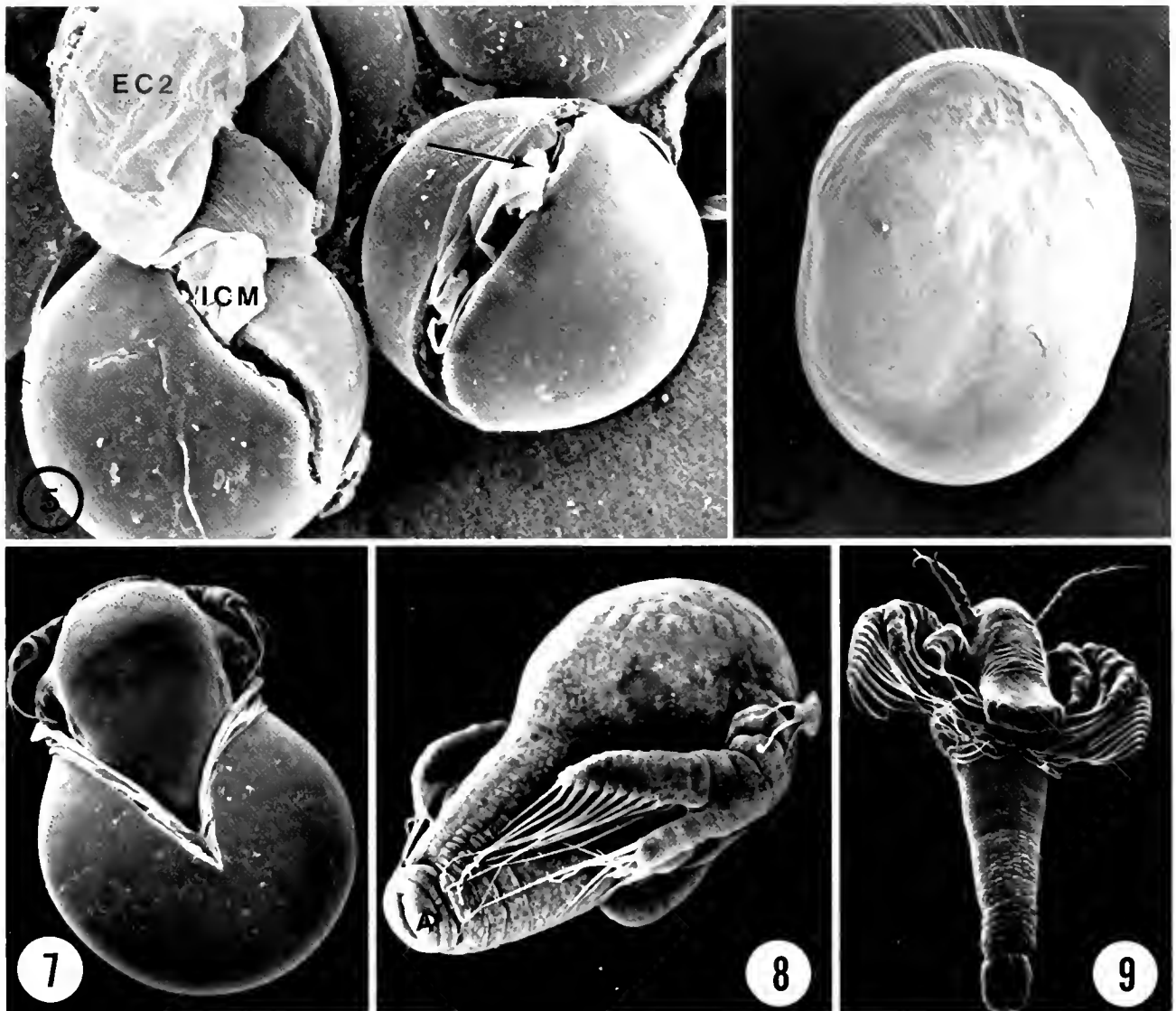
Figures 1–4. Figures 1–4 are scanning electron micrographs of brine shrimp prenauplii emerging or shells and cuticles from which brine shrimp have emerged. (Figure 1) Cysts attached to a glass coverslip with glue, three prenauplii emerging in synchrony. Glue remains where cysts have become unattached. The prenauplii emerge upward, presumably covered with two adhered membranes (ICM outside, EC2 inside). 95 \times . (Figure 2) Close up of emerging prenauplius. Note the straight edge of the shell and the serrated edge created by separation along polygonal plates of the EC1 (arrow). 400 \times . (Figure 3) The nauplius I has left the cyst shell and escaped from the EC2. Note the EC2 at the arrow has torn, apparently when it was adhered to the edge of separated polygonal plates. 475 \times . (Figure 4) Similar to Figure 3, but what may be a fragment of a polygonal plate (arrow) is attached to the EC2. 250 \times . Abbreviations: S, shell (tertiary envelope); EC1, first embryonic cuticle; EC2, second embryonic cuticle; ICM, inner cuticular membrane of the EC1; PN, prenauplius; G, glue added to adhere cysts to circular coverslip.

amined by SEM). The bagged nauplius, although out of the cyst shell, nonetheless could not escape (hatch). This fact was established by removing dozens of oval bags containing active nauplii as viewed with transmitted light, placing them in oxygenated hatching medium (with NaHCO_3), and watching them over a 24-h period or longer to see if they escaped from their bag (hatched). None did, and all eventually died, although in some cases, the nauplius was able to escape part way out of the bag and to swim around with the bag attached. The totally bagged nauplius was, however, able to differentiate into a fully formed nauplius I, as

evidenced by the completion of a continuous surface covering, of the EC3 (exoskeleton, see Figs. 32–34).

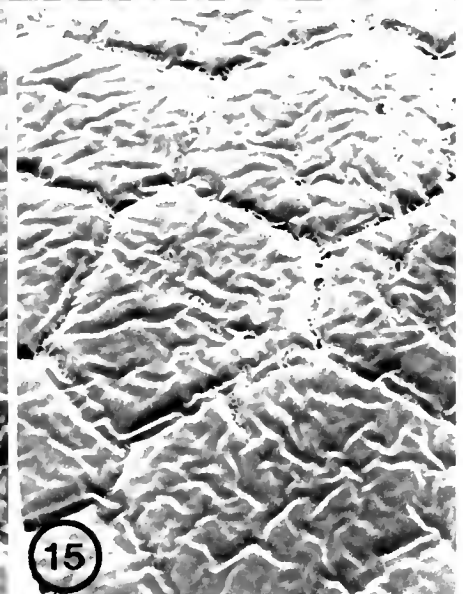
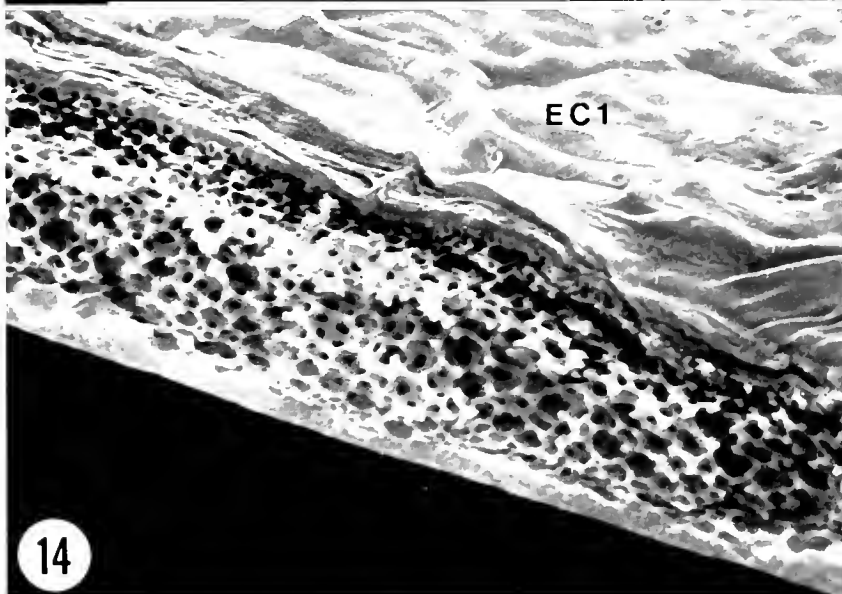
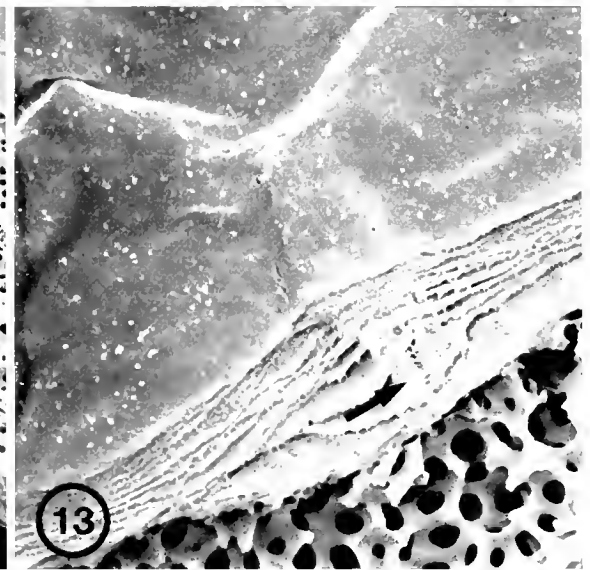
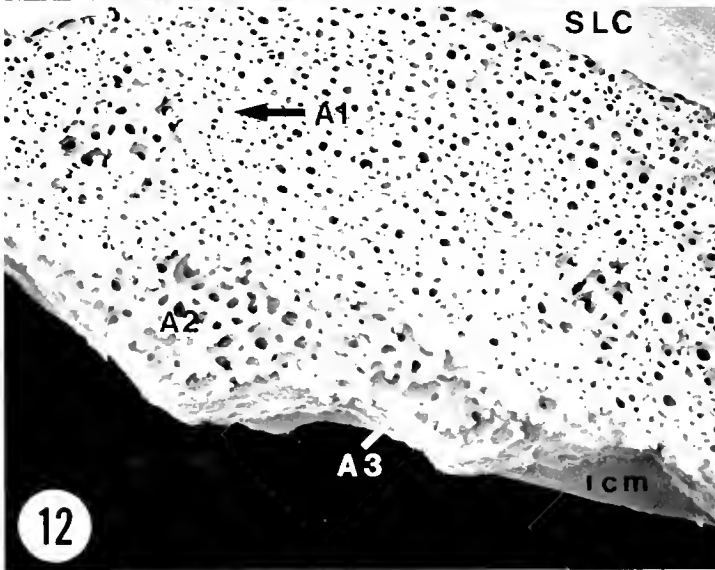
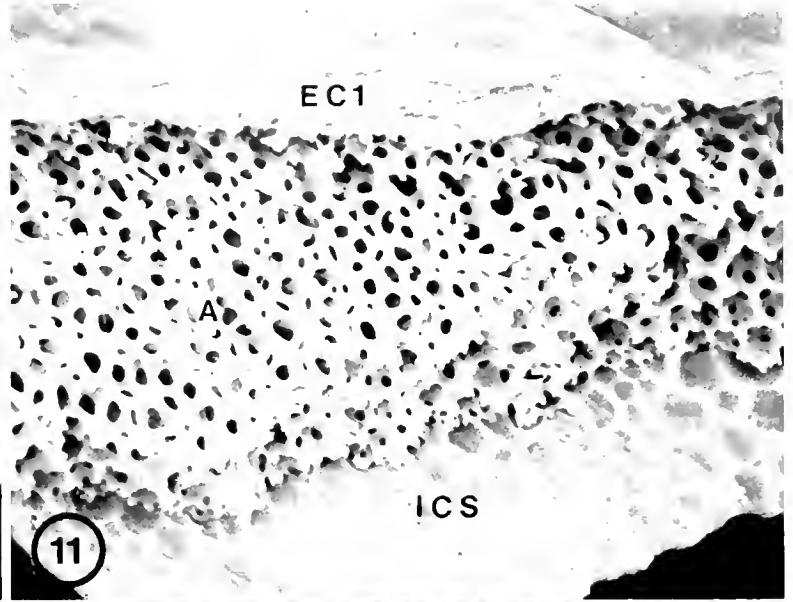
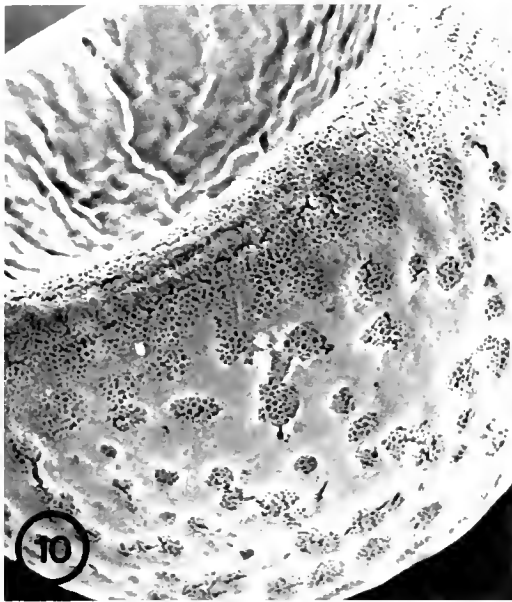
The Shell During Hatching: SEM Images

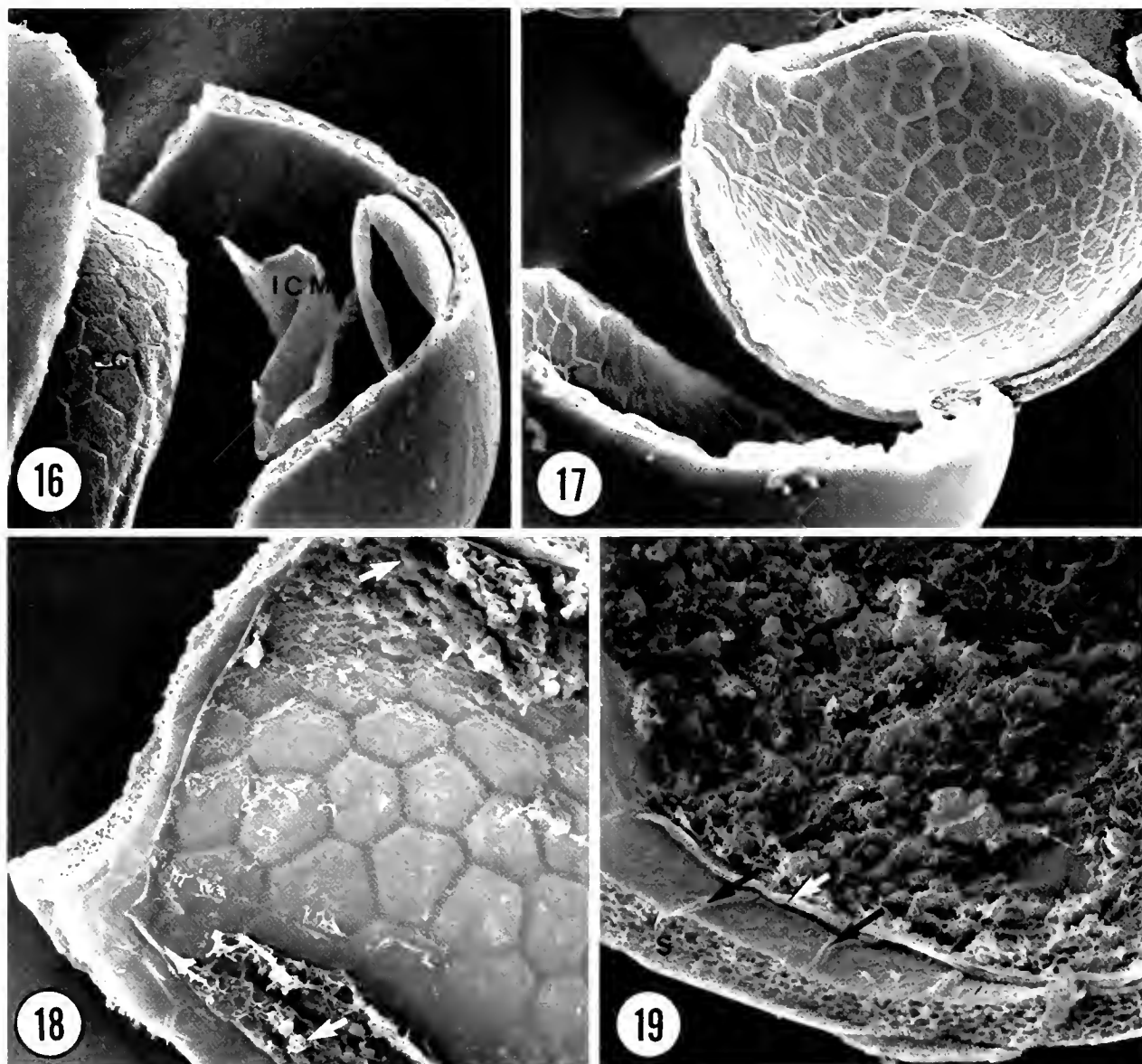
Whole cysts treated with a deshelling solution and examined early in this chemically induced degradation process illustrate the initial dissolution of the surface lamella and the adjacent cortical and alveolar subregions. The surface dissolution progresses as widening holes that eventually coalesce, exposing the alveolar subregions (Fig. 10). However, the most useful procedure for



Figures 5–9. Scanning electron micrographs of vacated cysts, prenauplii, and nauplii of brine shrimp. (Figure 5) Empty cyst shells, the left shell with the EC2 and ICM, the right shell with only the ICM (arrow, presumably the EC2 became detached by the emerging prenauplius). Note that the shells closed around the cuticles once the prenauplius emerged. 225 \times . (Figure 6) Oval-bagged nauplius, naturally emerged from its shell and presumably covered with the ICM and EC2. 230 \times . (Figure 7) Nauplius emerged halfway, naked, from unattached cyst; the ICM and EC2 remain within the shell but are visible near the shell edge, where they split prematurely to accommodate the emerging nauplius 1. This nauplius would have died because the shell holds it in place, and osmotic pressure cannot be generated to free it. 210 \times . (Figure 8) Newly emerged nauplius 1, in lateral view, that has escaped from the EC2. 110 \times . (Figure 9) Metanauplius 1 in ventral view. 55 \times . Abbreviations: EC1, first embryonic cuticle; EC2, second embryonic cuticle; ICM, inner cuticular membrane; A, anus, the slit to the left of "A."

Figures 10–15. SEM of the brine shrimp cyst shell (tertiary envelope) and the EC1. Figures 11–14 are of the shell and EC1. Figures 11–15 are CPD and fractured, before coating with heavy metal for SEM. (Figure 10) Dry (unimbibed) cyst briefly treated with deshelling solution to dissolve the shell and then CPD. The cortical region is eroded as circular areas revealing the alveolar region. (Figure 11) The EC1 is torn obliquely, revealing its lamellate layers. The alveolar region (A) is fractured and is missing from the pockmarked inner cortical surface (ICS) of the shell. (Figure 12) Fractured cyst shell attached to the EC1. Three alveolar subregions are evident (A1, A2, and A3). Outer surface of the ICM is evident. (Figure 13) Details of the lamellate EC1, in the region where polygonal plates articulate (arrow) along their rim, creating paired radial septa (see also Figs. 14 and 15). (Figure 14) Nearly transverse section of cyst shell and attached EC1, with a negative bas-relief of the inner surface of the EC1 polygonal plates. The EC1 has broken off with the TB revealing the alveolar subregions. (Figure 15) Outer surface of the EC1 with a positive bas-relief of polygonal plates, except at their rim interfaces, which are recessed. Plate wrinkles are artifactual. Abbreviations: SLC, surface lamella fused to cortex; EC1, first embryonic cuticle; ICS, inner cortical surface of the shell; ICM, inner cuticular membrane of the EC1; A1, first alveolar region; A2, second alveolar region; A3, third alveolar region.





Figures 16–19. SEM of CPD cysts of brine shrimp, all pressure fractured before coating with heavy metal for SEM. (Figure 16) Vacated cyst; the ECI has pulled away from the shell on the left. Note the positive bas-relief of polygonal plates on their outside surface. The ICM has pulled away from the shell on the right. (Figure 17) Empty shell; the ICM has pulled away from the ECI, exposing the inner surface of the polygonal plates in negative bas-relief. (Figure 18) Imbibed cyst with embryonic tissue attached. Primary electron beam penetration shows the polygonal surface of the ECI (still attached to the shell) as viewed through the plasma membrane of the embryo and the ICM. Note embryonic cells at white arrows and polygonal plate margins without seams. (Figure 19) Young postencystic embryo fractured through the shell and with more intact embryonic tissue than in Figure 18. Black arrows point to seam of adjacent polygonal plates of the ECI. Although the ICM is missing where polygonal plates are evident, the ICM is likely present at the white arrow, which points to the area of fusion of the inner ECI (including the ICM) with the embryonic surface of plasma membranes. Abbreviations: S, cyst shell (tertiary envelope); ICM, inner cuticular membrane of the ECI; ECI, first embryonic cuticle.

studying the internal features of the shell was to fracture CPD cyst shells that had been discarded by nauplius emergence. This sometimes revealed the innermost cuticle attached to the shell, the first embryonic cuticle (EC1). Fractures through the shell, when somewhat diagonal, showed the complexity of certain regions within the ECI by exposing broad surfaces at their regional interfaces. Although the outer lamella of commercially processed cysts does not separate from the cortical region by shell fracturing, the cortical region readily separates from the adjacent alveolar region, revealing its inner, pockmarked surface with a wide range in size of depressions (Fig. 11). Of the three alveolar subregions previously

defined (Lee et al. 1994), the A1 and A2 are clearly distinguished here (Fig. 12), but the innermost subregion, the A3, is barely discernable.

Fractured cysts show the ECI surface of polygonal plates attached to the shell and the ICM of the ECI. The ECI is the innermost cuticle distinguishable by SEM. A close view facing toward the inner surface of the ECI in fractured whole cysts shows layering within this cuticle. These lamellae are periodically fused by obscure perpendicular connections (the radial septa of TEM), which create the edge material delimiting polygonal plates (Figs. 13 and 14). Chemical dehydration followed by CPD of empty cyst

shells causes some collapse of the EC1, so that the polygonal plates have a negative bas-relief (the plate edges protrude internally while the plate surface appears sunken), whereas the polygonal plates have a positive bas-relief when viewed from their outer surface (and the plate margins then appear recessed; Figs. 15 and 16). When empty cyst shells are fractured, the shell and the EC1 sometimes separate revealing the outer and inner surfaces of the EC1, in which case the outer surface is recognized by being in positive bas-relief (Fig. 16). However, there can be two morphologies for the inner surface, depending on whether or not the ICM is still part of the EC1 or if it has pulled away. For example, when the ICM is missing (pulled out and then off during nauplius emergence), the lamellar material of the EC1 collapses with specimen preparation so that the polygonal plates appear in negative bas-relief (recessed) and the areas of articulation of the plate margins are clearly protruding (Fig. 17). However, when the ICM is still attached to the rest of the EC1, then the ICM surface is smooth (in CPD preparations; Fig. 16). Because of primary electron beam penetration in SEM, viewing of polygonal plates through the embryonic tissues and membranes is possible (Fig. 18). In such cases, the polygonal plate margin pairs always appear as single, dark bands (rather than paired with a seam when they shrink), forming a reticulum and having no surface relief, positive or negative (Fig. 18). The thinness of the embryonic membranes and cuticles makes it impossible, in some examples, to distinguish among them by SEM. However, when negative bas-relief polygonal plates are evident, and the seam of the separate plate margins is revealed (Fig. 19), our interpretation is that the ICM is missing in that area to allow for distinct resolution of these paired edges.

The Shell: TEM Images

There is an outer covering over the cortical region of the shell (Fig. 20, see Fig. 29), a distinct surface lamella less electron dense than the cortical region and tightly bound to it. In glancing thin sections, this lamella appears diffuse rather than well defined, as in median transverse sections, but it is always without pores. However, the midarea of the cortical region is penetrated by elongated, mostly radially aligned pores or channels (Fig. 20, see Fig. 29), also termed aeropyles (Anderson et al. 1970). In transverse sections, these pores appear in places to be arranged in a scalloped pattern rather than in a straight row (Fig. 20), a pattern that reflects (parallels) the outer surface curvature of the large pores of the alveolar region. Both SEM and TEM images suggest that the cortical region is not tightly bound to the alveolar region because connections to it are narrow and infrequent when examined in transverse section. Therefore, the cortical/alveolar interface easily fractures in normal specimen preparation (Fig. 20, see Fig. 29) and when pressure is applied to the shell for the purpose of exposing its internal features (Fig. 11).

The alveolar region is found interior to the cortical region and consists of three subregions designated as A1 (outer), A2 (middle), and A3 (inner) (Lee et al. 1994). The A1 subregion is sandwiched between the cortical region and the A2 subregion. It is thin and flat with interconnecting arms around small pores, creating a lacy appearance (by SEM). The connecting arms of the A1 to the cortical region (Fig. 20) are not nearly as extensive as the connections to the A2 subregion (Fig. 20). The A1, with its small pores, serves as the transitional subregion to the more open (large pores) and much thicker A2 subregion.

The A2 subregion is the major porous region of the shell, with spherical to oval chambers created within it and delimited by

armed processes (Fig. 20). Usually about five or six arms interconnect around a pore (Figs. 20, 21). The largest pores, and their continuous open interconnections, are best visualized by comparing TEM (Figs. 20 and 21, see Fig. 29) and SEM images (Figs. 11–14). There are elongated, thin pores or flat channels that appear within the arms of this subregion (Fig. 20, see Fig. 29), but they are not as numerous as those in the innermost alveolar subregion (A3).

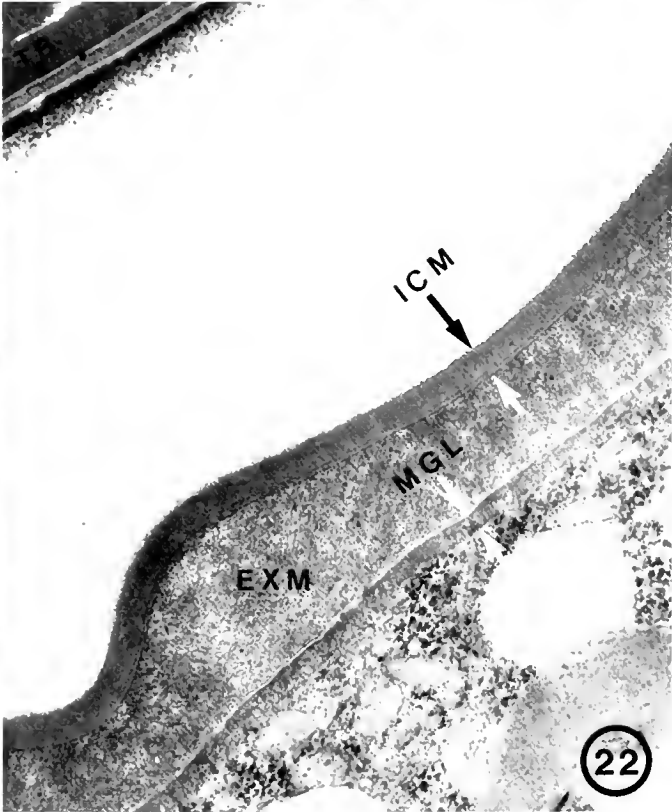
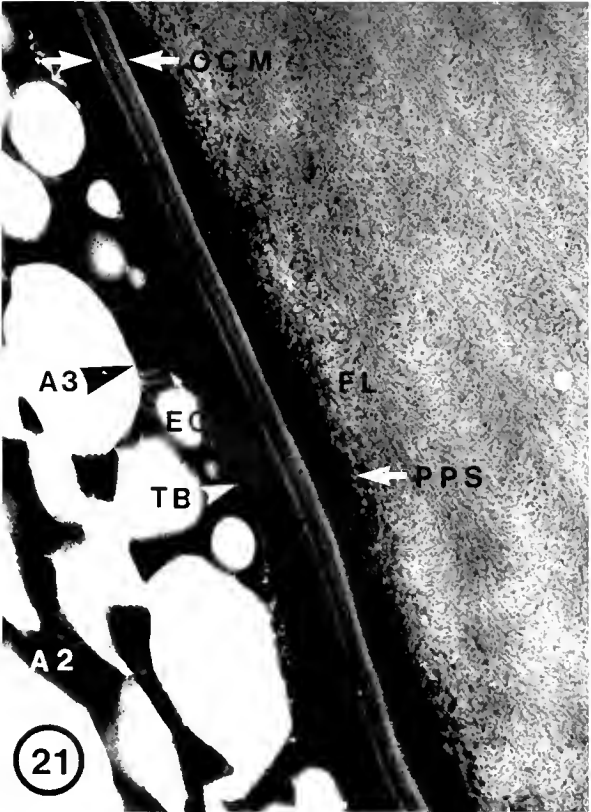
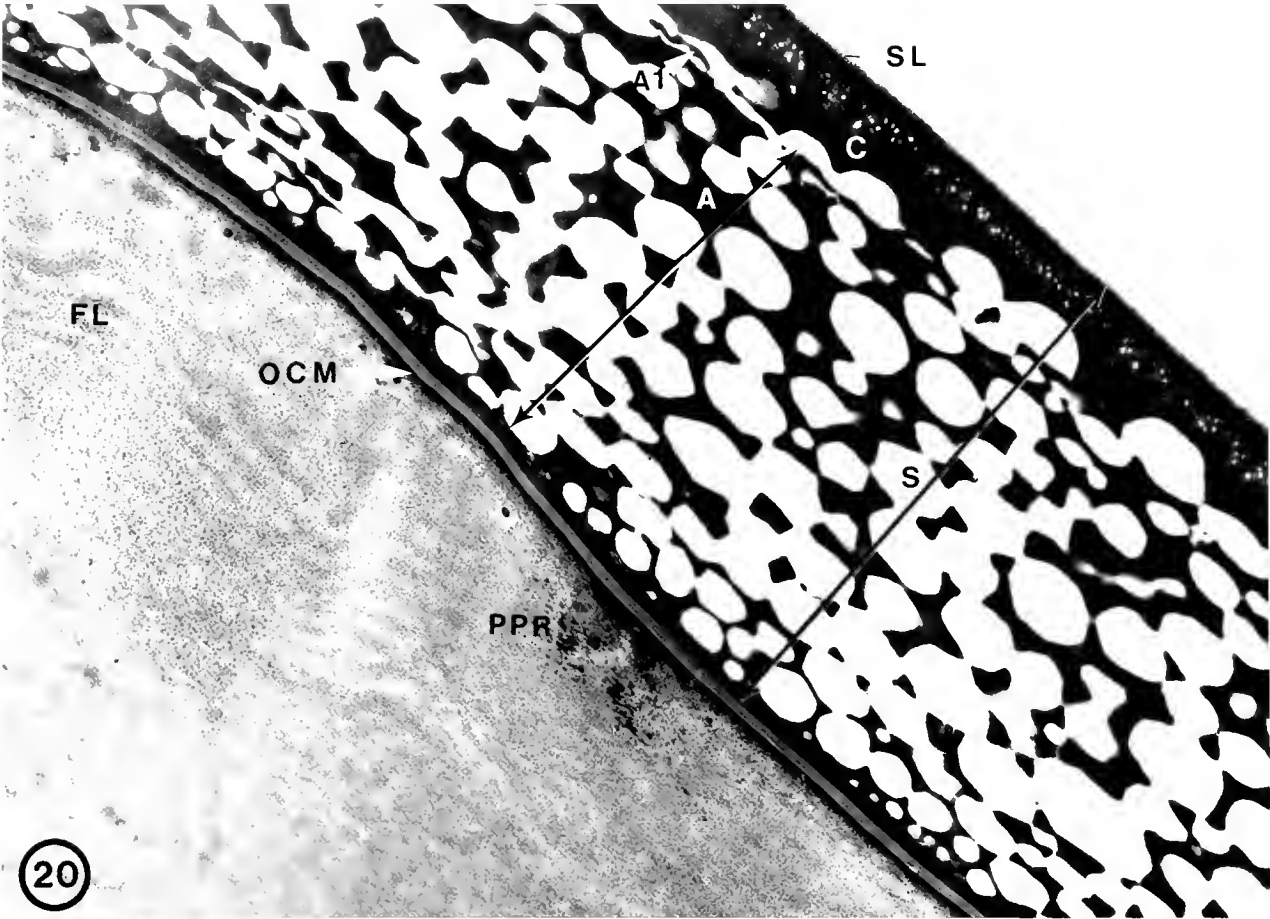
The A3 subregion is very thin and, like the A1, is a transition to the A2. It also has open, oval pores formed by interconnecting armlike processes, but the open areas are much smaller than even those of the A1. Also, unlike the A1 subregion, there are many tiny, elongated pores in this layer that appear as flat channels, oriented mostly perpendicular or oblique to the tertiary envelope base (TB; Fig. 21) and spanning the entire A3 subregion. That is, these channels run continuously from the top to the bottom of this subregion. In addition, where they are also parallel to its surface, they serve as a potential fracture line and visually delimit the inner surface of the A3 (Fig. 21, see Fig. 29).

Of similar electron density as the material of the cortical and alveolar subregions, the innermost layer of the shell, the TB, is thin but, in contrast to the cortical region, is typically without pores or channels. This layer is always firmly attached to the OCM of the EC1. That is, gaps between the TB and OCM were never observed, except when cysts were chemically treated to dissolve the TB (see Fig. 35).

The Embryonic Cuticles: EC1, EC2, EC3, Mostly From TEM Images

Attached to the TB of the cyst shell is the first embryonic cuticle (EC1), which is composed of a broad multilamellar region sandwiched between the much thinner OCM and the ICM, forming a tripartite structure. The broad, multilamellar middle of the EC1 is sometimes thicker than the shell itself (Fig. 20), and the lamellations are often difficult to demonstrate without overstaining or overdevelopment of prints. The OCM is ultrastructurally the most complex of the membranes within the cyst (most number of distinct layers as dark or clear regions), considering those present before and after hydration and complete postencystic development (Fig. 21). However, to demonstrate this complexity and further layering requires higher magnification than shown here. In thin sections, periodically, there are ill-defined ridges perpendicular to the innermost surface of the OCM, and they occur in pairs constituting septa (Fig. 20, see Figs. 26 and 29). These septa create polygonal reticulations in surface view (by SEM or transmitted light microscopy) and are more electron dense than the fibrous material that composes the remaining material of this lamellate region. The polygonal plates are composed of fibrous, wavy concentric layers (Fig. 20) that are barely detectable (Figs. 21 and 22) or undetectable by TEM (see Fig. 29), but are quite evident by SEM (Figs. 13 and 14). Even within the same cyst, as examined by SEM, the number of these layers either varies in number or else fails to separate sufficiently to reveal their presence. By SEM, we differentiate many layers within the EC1 (Fig. 13), more than we demonstrated by TEM (Fig. 20). The radial septa of the EC1 appear continuous, with a thinner, dark-staining material adjacent to the OCM, and thus we refer to this entire, continuous layer as the "polygonal plate surface." As far as we could tell, this surface layer of the EC1 is never exposed in specimen preparation because it is always fused to the surrounding OCM.

The innermost layer of the tripartite EC1, the ICM, like the OCM, is characterized by uniformity in thickness. The ICM is also characterized by having a double line delimiting its innermost



surface (Figs. 22–25). The outer surface of the ICM is finely fibrillar; the fibrils are often perpendicular and loose, creating a fuzzy appearance in transverse view (Fig. 22; see Fig. 34).

Membrane Development Within the Postcystic Embryo

In cysts that have the earliest surface evidence of embryo differentiation, a finely granular extracellular matrix appears between the EC1 and the embryo surface. The embryonic surface at this stage (Fig. 22) is composed only of confluent (fused) epidermal cell plasma membranes. Toward the inside of the plasma membrane is also a finely granular material, similar in morphology and electron density to the material outside this membrane (Figs. 22 and 23). Unlike the extracellular matrix, which is highly variable in thickness and typically fills whatever space is available (Fig. 23), this intracellular, fine granular material is more uniform in thickness, appearing as a distinct band just internal to the plasma membrane at its most peripheral surface (Figs. 22 and 23). Because of the position of this finely granular, intracellular matrix, it is likely to give rise to the finely granular extracellular material that appears during development when the intracellular matrix disappears. This intracellular layer in the outer periphery of the epidermal cells becomes uneven in thickness when the embryo begins to deeply cleave during the formation of paired appendages, but it is still recognizable as a distinct layer (Fig. 23).

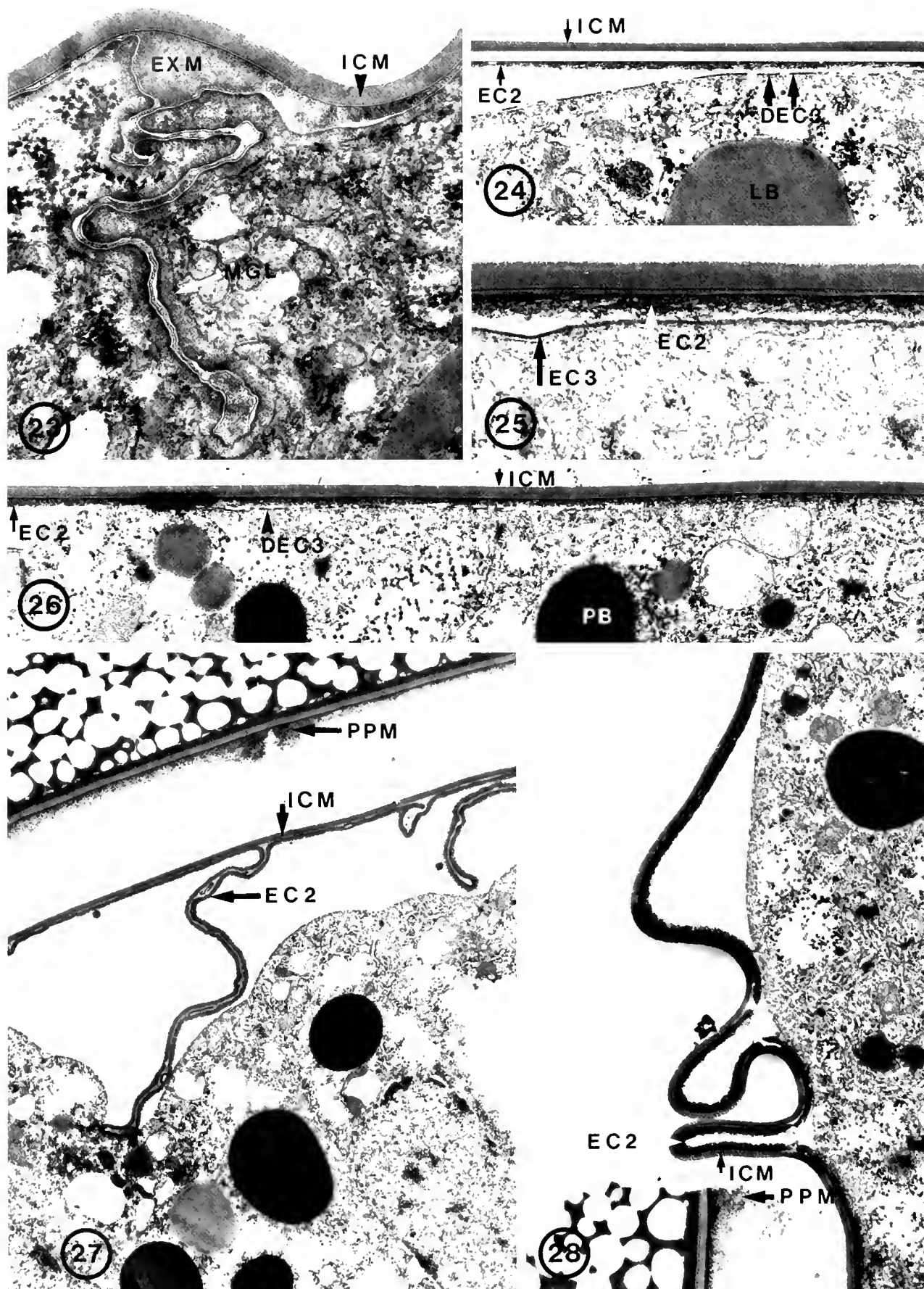
In embryos that undergo postcystic development (incubated in aerated saltwater), the first new layer to differentiate is the second embryonic cuticle (EC2), also known as the hatching membrane (cuticle). This highly fibrous cuticle (see Figs. 30 and 31) is initially oppressed to the inner ICM surface and therefore would be of the same surface area as the ICM, which is determined, and fixed in size, by the time the cyst is released by the female. The EC2 is tightly adhered to the ICM, as evidenced by the ICM and the EC2 remaining together even when the prenauplius surface developmentally moves away from the ICM surface (Figs. 24–28) as a result of the embryo changing shape during development within the shell. When the surface of the embryo increases in size as paired appendages differentiate, so does the EC2 proliferate on this newly expanding embryo surface while remaining continuous with that portion of the EC2 still adhered to the ICM. That is, the EC2, which is adhered to the inside of the shell, is of a fixed surface area where attached to the shell while still being dynamic in that it increases in overall size along with the embryo surface on which it forms within the unhatched cyst. However, as mentioned previously, the ICM has no further addition to its membrane surface after the cyst enters diapause and then breaks diapause and becomes hydrated.

The EC2 is the first cuticle produced during postcystic development, and the next is the exoskeleton of the prenauplius, known as the third embryonic cuticle (EC3). In all examples ob-

served, the EC3 was incompletely formed before the prenauplius emerged from the cyst shell, as evidenced by interrupted dark-line segments interpreted to be exterior to the prenauplius plasma membrane surface and thus the immature EC3 surface (Figs. 24, 25, and 27–30). However, when emerged from the shell (within the EC2 or within the ICM + EC2 in the oval bag configuration), the embryo had a continuous (fully formed), thin, exoskeleton (Figs. 25, see Figs. 32–34), and thus was interpreted to be a nauplius 1.

It was noted that even before the EC3 surface is continuous, fine, granular material remains between the EC2 and the ICM. This fine granular matrix, although in many places collectively as dense as membrane material, is not considered membranous because it tapers while the true membranes alongside it do not (Fig. 30, the ICM and EC2). Two other examples of tapering of fine, granular material between the ICM and the EC2 are illustrated here (see Figs. 32 and 33). The tapered material becomes diffuse at its thickest region, adding further support to the interpretation that this material is not membranous; however, much of it may be compacted. Nonetheless, the granular material between the ICM and the EC2 does maintain a layered appearance within the ICM and the EC2 as prenauplii are emerging in what will be an aborted hatch attempt (emergence method two); apparently, this material can be trapped there to the extent that it is not lost in the sequence of fluids used to chemically dehydrate the sample for TEM (Figs. 30 and 31). It should be noted that the EC2 has a single line delimiting its outer surface, which is often wavy (Figs. 30 and 31), supporting the SEM observations that it wrinkles easily (Figs. 3–5), whereas the ICM has a single line delimiting its inner surface (Figs. 30 and 31) and is never wavy. As noted previously, in the third method of emergence (induced here by leaving NaHCO_3 out of the hatching medium), when the shell breaks and the prenauplius emerges, the pair of adhered cuticular membranes (ICM + EC2) are not left within the shell, i.e., they emerge together, totally abandoning the shell and creating a stiff oval bag surrounding the prenauplius, or nauplius 1 (Fig. 28). Because this bag configuration had not been previously described ultrastructurally, and perhaps not even recognized in some cases as being different from the tapered bag (parachute), we sectioned a nauplius 1 in a bag and examined its entire perimeter. This bag type, also documented by SEM (Fig. 6), turned out to have three transverse section morphologies, depending on the position around the nauplius 1 that was observed. The outside cuticle was not wrinkled, as is typical of the EC2 hatching cuticle, but was smooth and firm because the outer cuticle was the ICM rather than the EC2 (Fig. 32). Elsewhere around the nauplius, the EC2, although attached in some regions to the ICM (Fig. 32), became detached elsewhere (Fig. 32) and repeatedly folded back on itself (Fig. 33). Finally, in still other areas, the EC2 had pulled away from the ICM and the nauplius surface so that the ICM alone covered the nauplius (Fig. 34). It appears

Figures 20–22. Transmission electron micrographs of brine shrimp cyst shells, from cysts that cracked open during prenauplii emergence (Figs. 20 and 21) or were in the process of early postcystic development (Fig. 22) when fixed. (Figure 20) Cyst shell (tertiary envelope) with the EC1 attached (innermost portion, the ICM not shown). (Figure 21) Details of the interface between the TB and the OCM (between the white arrows); also shows the fibrous middle and outer regions of the EC1. (Figure 22) Stage of postcystic embryonic development in which the embryo secretes fine, granular material between it and the surrounding ICM of the EC1. A peripheral, intracellular area below the plasma membrane (between white arrowheads) appears to be the source of the fine, extracellular granular material. 17,000 \times . Abbreviations: S, cyst shell (tertiary envelope); SL, surface lamella of cyst shell; C, cortical region of shell; A, alveolar region of shell; A1, A2, and A3, the A subregions; EC, elongated channel of the A3 subregion (small white arrow); TB, tertiary base of cyst shell; OCM, outer cuticular membrane of the EC1; PPS, polygonal plate surface; PPR, polygonal plate rim (always in pairs); FL, fibrous layer of the EC1, lamellate; EC1, first embryonic cuticle; ICM, inner cuticular membrane (between black and white arrows) of the EC1; EXM, fine extracellular matrix; MGL, matrix generation layer.



then that although both membranes emerge together in the oval-bag configuration, perhaps the intense stroking activity of the antennae and antennules of the nauplius 1 breaks the inner membrane (EC2) but is usually unable to break the outer membrane (ICM) of this bag; thus, the nauplius 1 dies inside the bag.

The "Deshelled" Cyst Surface

Finally, in order to ascertain the surface material of cysts treated with deshelling solution, whole treated cysts were examined in thin section by TEM. These treated cysts were orange in color before fixation, and we assumed that the shell had been chemically dissolved, because SEM examination of these cysts failed to suggest that any shell remained. In fact, although we normally get orange cysts within 10 min of treatment in the deshelling solution (while the untreated cyst with shell is generally reddish-brown), we increased the time to 30 min to increase the likelihood that the shell was totally dissolved. However, TEM examination of the 30-min "deshelled" cysts showed that the TB largely remained firmly attached to the OCM and that the degree of shell dissolution, as determined by examining the entire circumference of a cyst, was only slightly variable (Figs. 35 and 36). Two small areas where the TB was missing (Fig. 35), so that the OCM was the outer surface (as expected), might have been a result of the TB being cracked off the surface during rehydration of the "deshelled" cyst. However, some dissolution within the OCM argues against this being the only reason why the TB is missing in some areas.

DISCUSSION

It has been 30 years since the first report on the ultrastructure of the cyst and postencystic membranes that develop in the brine shrimp *A. franciscana* Kellogg (Morris and Afzelius 1967). Although our study reveals no new ultrastructural features of the cyst shell, it clarifies some previously studied or unstudied features of membrane and cuticle development. Major features of diapause-broken cysts (postdiapause, quiescent stage) and those that have imbibed water, leading to prenauplii emergence and hatching, are summarized at the end of this discussion and in the final figure (Fig. 36). Most important, we have reviewed the earlier data from light microscopy relevant to postdiapause cysts and their development into nauplii that hatch or fail to hatch. Also, we have integrated these observations into the context of our ultrastructural data while incorporating more recent terminology.

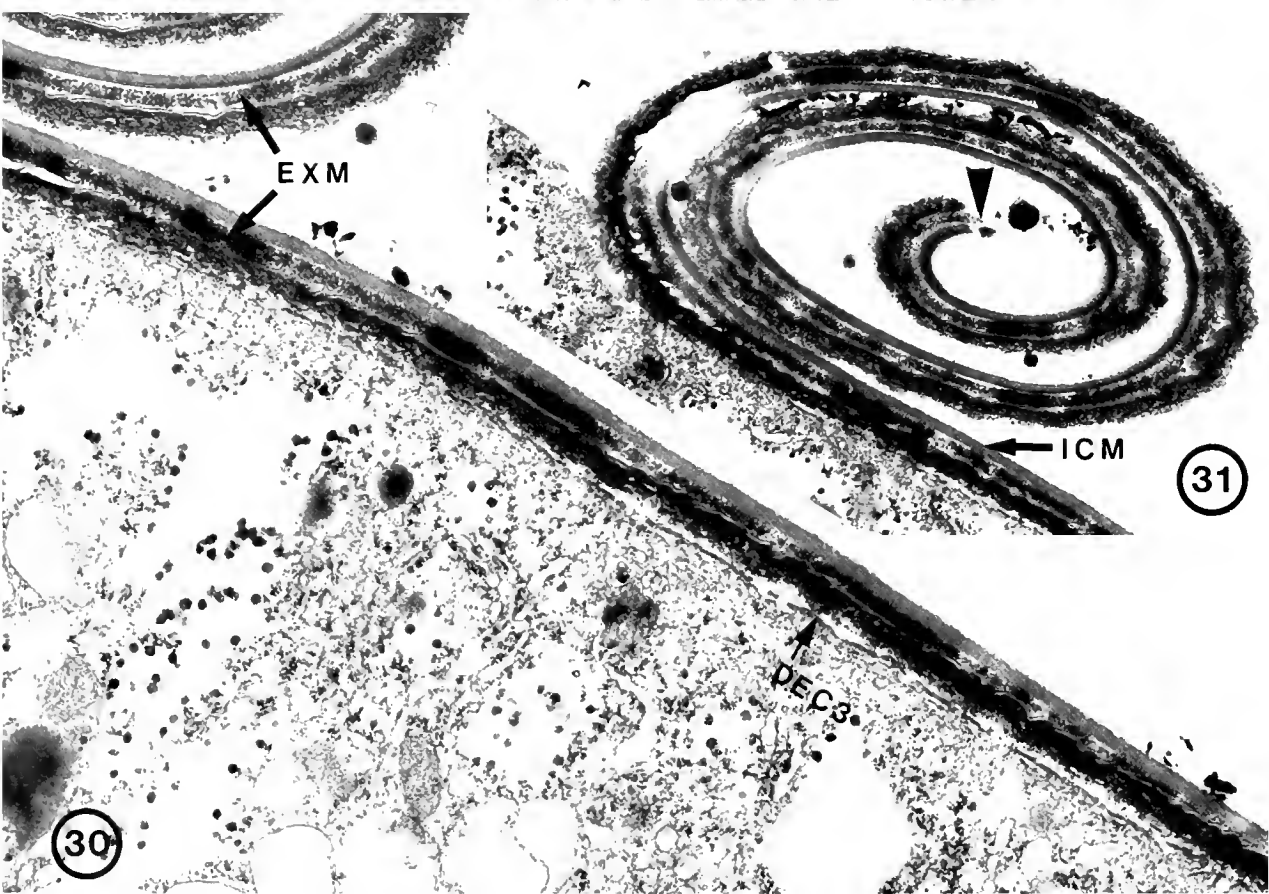
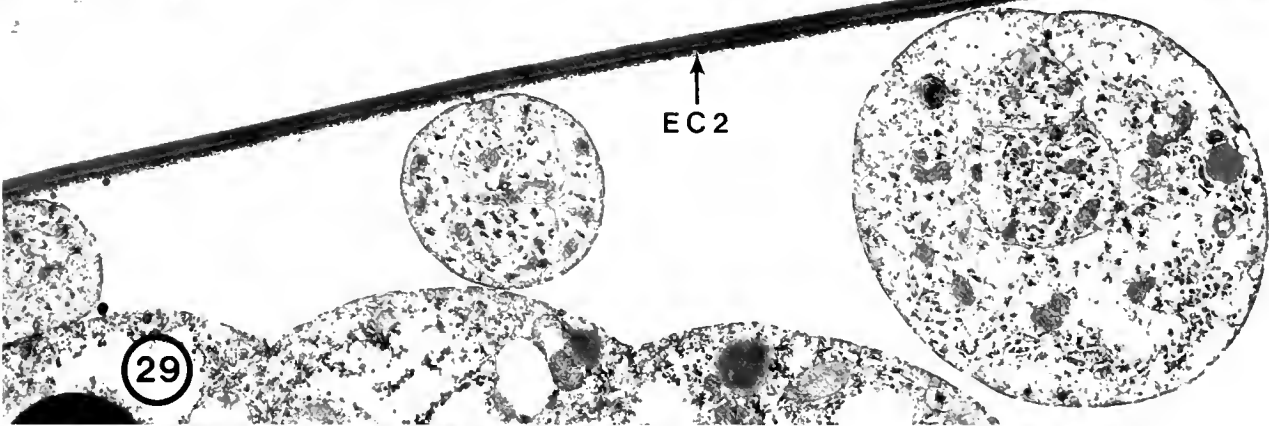
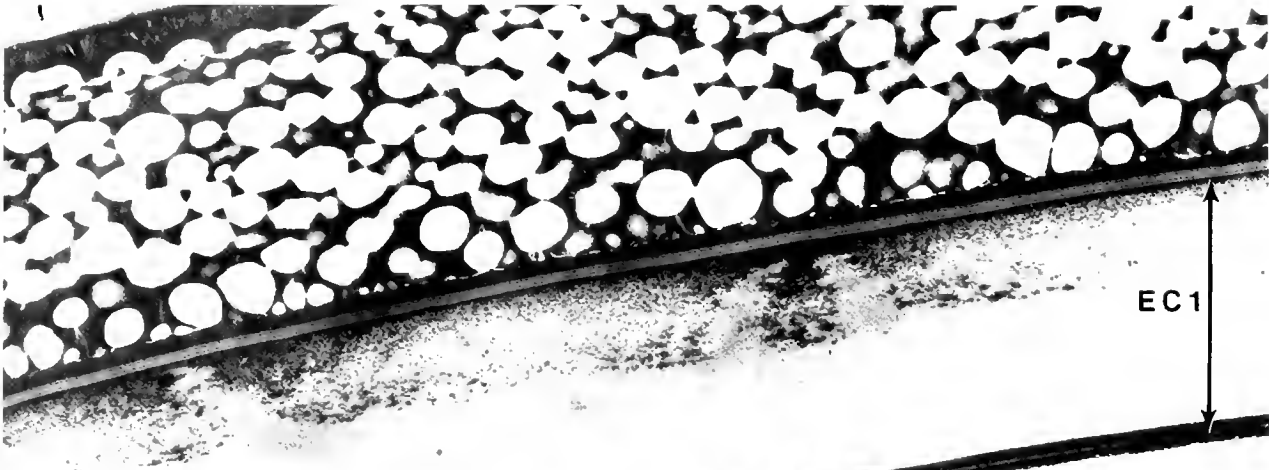
The postencystic embryo differentiates into a prenauplius before hatching, on imbibition of hatching medium by the highly

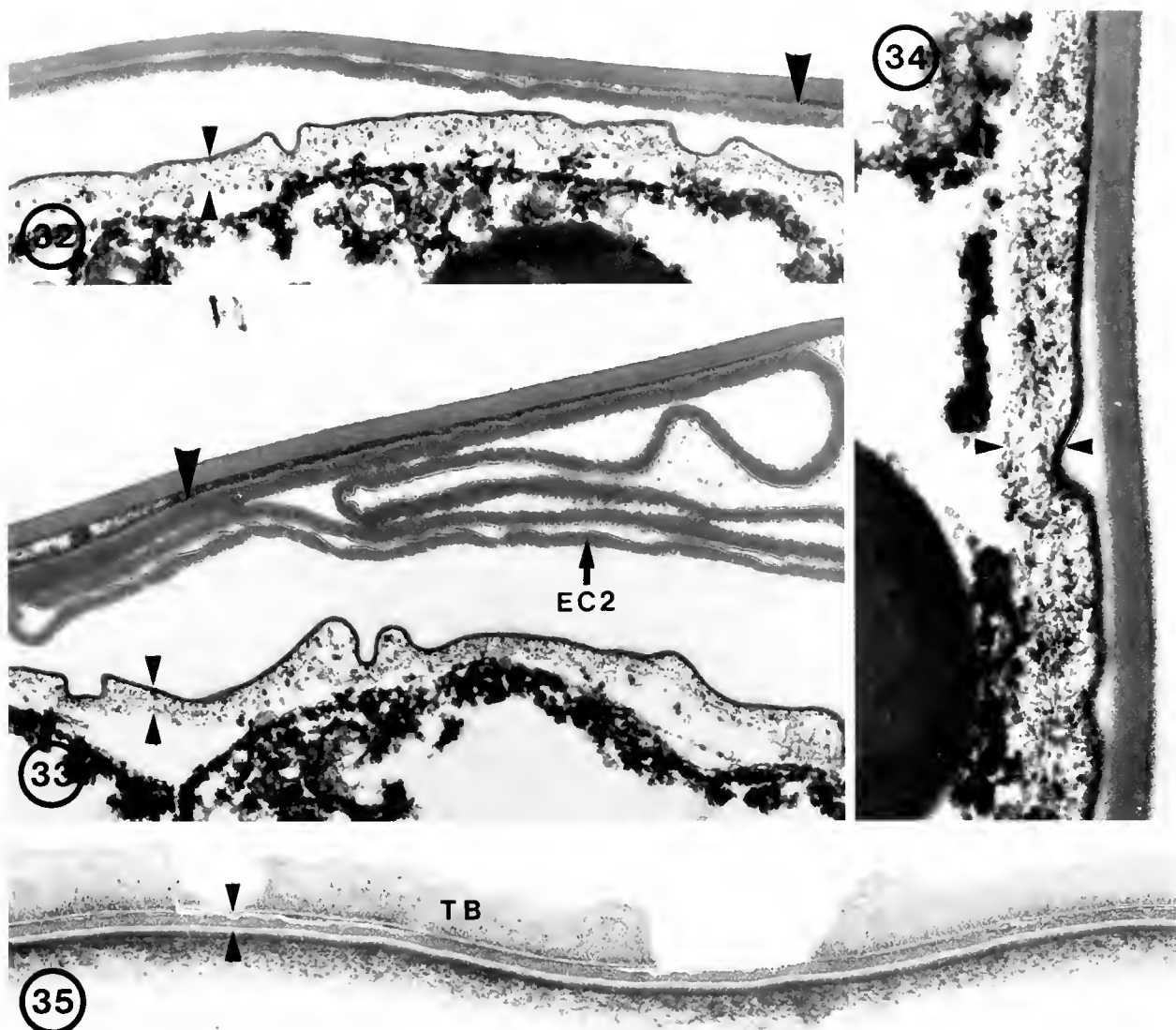
porous shell, followed by water uptake into cells of the embryo. During early postencystic development, the EC2 becomes the first new cuticle produced by the embryo and does not develop from the ICM, as sometimes interpreted (Van Stappen 1996). That is, there are no additions to the EC1 or its ICM after diapause. By late postencystic development, the head region becomes segmented, with paired appendages, the antennules and antennae (Fig. 29) being most easily recognized. As these paired appendages begin to differentiate, they greatly increase the surface area of the prenauplius developing within the cyst shell. The hatching cuticle (EC2) apparently continues to be produced throughout differentiation, as evidenced by folded (loose) cuticle in the extracellular region around the prenauplius within the cyst shell (Fig. 27). This additional folded cuticle is produced by surface epidermal cells of the embryo that develop away from the inner shell surface during postencystic development (cf. Figs. 23 and 27). In this way, the EC2 attains a greater surface area than the ICM to which part of it is attached. Again, this is because development of the ICM portion associated with the inner shell surface forms only while the embryo is retained within the female and before diapause. Eventually, the loose EC2 beyond that adhered to the ICM is inflated through water uptake. Part of the EC2 originally adhered to the ICM may remain attached at the caudal end, if the prenauplius emerges from the shell covered only with the EC2 (the typical method of emergence followed by hatching).

Because the developing embryo and prenauplius are so large, it was possible to section only one individual at a time, for examination by TEM. In order to ensure suitable penetration of fixing, dehydrating, and embedding materials for TEM, cysts were cut in half at an early developmental stage to allow for penetration of these agents, or only water-imbibed cysts that were cracking open due to water uptake and development were processed. In this way, we could view cuticles and membranes that were still within the cyst shell, or that had emerged with the prenauplius, and note membrane diversity and cuticle configurations during postencystic development. Cyst shell fragments from hydrated cysts (produced by applying pressure with a razor blade to CPD cysts) provided views from within the embryo (Figs. 18 and 19). The developing EC2, the ICM, and the fibrous region of the EC1 were sufficiently electron transparent to reveal the denser polygonal plates of the outer fibrous layer, through those layers from the embryo side (Fig. 18).

With regard to the earliest literature on the cuticular details of hatching, Myint (1956) forced 10- to 12-h-old prenauplii out of their shells prematurely and documented two membranes around

Figures 23–28. Transmission electron micrographs of postencystic embryos and prenauplii of brine shrimp in development. (Figure 23) Two adjacent epidermal cells of the prenauplius separated by a fine, granular, extracellular matrix continuous with that just within the ICM (labeled EXM). The layer producing this matrix appears to be the intracellular band (white arrow) of similar staining material nearest the plasma membranes of these adjacent cells. 21,600 \times . (Figure 24) Developing prenauplius has produced the fibrous EC2, which is separated on the left from the prenauplius epidermis composed of an immature EC3 surface (dark broken lines at arrows). 13,850 \times . (Figure 25) Fibrous EC2 is seen beyond the continuous EC3 surface. 29,000 \times . (Figure 26) Surface of prenauplius covered with the uniformly thick and homogeneous ICM and the less uniformly thick and fibrous EC2 (hatching cuticle). The exoskeleton (DEC3) is discontinuous (surface is immature). 14,450 \times . (Figure 27) In areas where the EC2 remains within the shell, portions of it develop away from the ICM, on the embryo surface, during differentiation of the paired appendages of the prenauplius. 6,300 \times . (Figure 28) A prenauplius emerging from the shell. The ICM and EC2 are fused and appressed against the prenauplius within the shell, but folded and loose (detached) over the prenauplius outside the shell. Note half-septum (one polygonal plate margin as opposed to a pair) in the EC1, which suggests that the shell has split at the seam of previously adjacent polygonal plates. 8,550 \times . Abbreviations: EXM, fine, granular extracellular matrix; MGL, extracellular matrix generation layer (intracellular in origin); EC1, first embryonic cuticle; PPM, margin of a polygonal plate of the EC1; ICM, inner cuticular membrane of the EC1; EC2, second embryonic cuticle; DEC3, discontinuous (immature) exoskeleton surface; EC3, third embryonic cuticle surface (nauplius 1 exoskeleton surface); PB, protein body; LB, lipid body.





Figures 32–35. Membranes of a single, oval-bagged prenauplius of a brine shrimp that had naturally emerged from its shell (cf. Fig. 6). Figures 32–34 show the mature nauplius 1 exoskeleton (between arrowheads). (Figure 32) The exoskeleton is completely formed (continuous and mature), and the surrounding “membrane” is in fact the ICM (of the EC1) and the EC2, which are separated at the large arrowhead by a thin but dense extracellular granular matrix. The third embryonic cuticle (EC3) is the layer between the arrowheads, 15,000 \times . (Figure 33) There is dense, extracellular matrix material (large arrowhead) between the ICM and the EC2. Infolding of the EC2 has occurred, with its outer surface always identifiable because of the line edge, often wavy, which delimits its exterior surface. The exoskeleton (third embryonic cuticle) is the layer between the two arrowheads, 14,000 \times . (Figure 34) The EC2 is missing from this region (pulled away) so that the ICM (the band to the right of the right arrowhead) becomes directly oppressed to the third embryonic cuticle (between the arrowheads). This thin section is mostly oblique to the surrounding membranes, except at the right arrowhead, where the EC3 surface shows a line edge delimiting its exterior, 25,000 \times . (Figure 35) A cyst treated in a deshelling solution shows the TB of the shell still attached to the OCM (between arrowheads), except in two areas. Note the dissolution and/or separation of the OCM. The bottom arrowhead spans the region below the OCM composing the polygonal plate surface, 24,850 \times . Abbreviations: TB, tertiary base of shell; EC2, second embryonic cuticle (hatching cuticle or parachute).

Figures 29–31. Transmission electron micrographs of brine shrimp cyst shells cracked open with the prenauplius partially emerged. Prenauplii in Figures 30 and 31 would not have lived because they would be trapped by the shell. (Figure 29) Cracked-open cyst shell with prenauplius paired appendages differentiated. The EC2 is tightly adhered to the ICM of the EC1. The EC3 surface is incompletely formed over the prenauplius body and appendages (broken dark lines on the surface), 7,925 \times . (Figure 30) Surface of an emerged portion of the prenauplius (aborted emergence). The EC3 surface is incompletely formed (immature); exterior to it is a fibrous EC2, and between it and the ICM is a fine, extracellular granular matrix that disappears from left to right, 18,350 \times . (Figure 31) Prenauplius has partially emerged; the ICM and EC2 are curled at the broken edge (cf. Fig. 7) and are separated by a finely granular EXM. The extracellular matrix granules between the ICM and EC2 are held together, membrane like, at the black arrowhead, 13,275 \times . Abbreviations: EC1, first embryonic cuticle; ICM, inner cuticular membrane of the EC1; EXM, extracellular granular matrix; EC2, second embryonic cuticle; DEC3, discontinuous (immature) third embryonic cuticle surface.

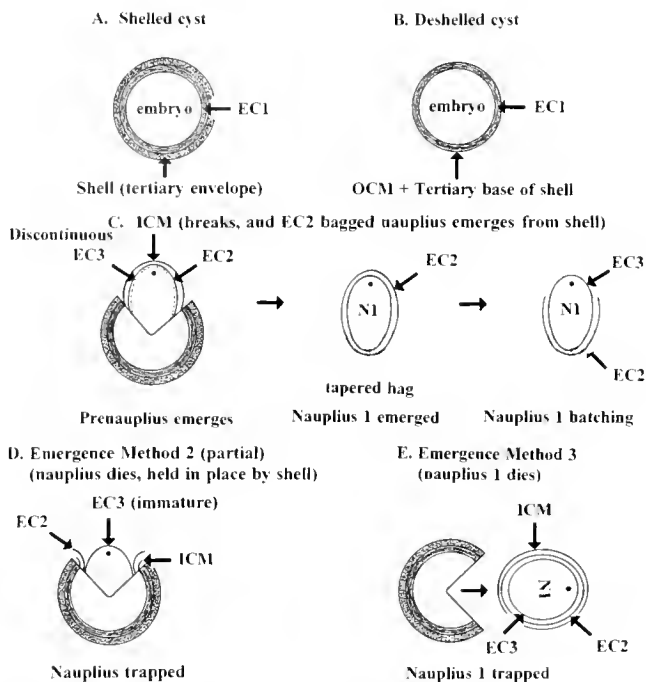


Figure 36. Summary of cyst features and methods of nauplius emergence and hatching in the brine shrimp *A. franciscana* Kellogg. (A) Shelled cyst. (B) Deshelled cyst. (C) Emergence method 1 (route to hatching). (D) Emergence method 2 (partial); (nauplius dies, held in place by shell). (E) Emergence method 3 (nauplius 1 dies). Abbreviations: N1, Nauplius 1; EC1, first embryonic cuticle; OCM, outer cuticular membrane of the EC1; ICM, inner cuticular membrane of the EC1; EC3, third embryonic cuticle, the exoskeleton.

them ("outer" and "inner"). Then, he showed that with natural hatching (not physically forced), the outer of the two membranes (ICM, in present terminology) typically was left behind. However, the double membrane-covered prenauplii illustrated by Myint (1956, Figs. 13 and 14) are tapered, that is, not oval like those experimentally produced by Sato (1967a, 1967b) or those produced in this study using Sato's technique. The fact that two membranes were around a tapered prenauplius (Myint 1956), and were also claimed to be present in an oval configuration (Sato 1967a, 1967b), has undoubtedly led to confusion. Perhaps many have either chosen to ignore both of those studies or have not been concerned about the number of membranes around a prenauplius. Nonetheless, Sato (1967a, 1967b) showed that by leaving NaHCO_3 out of the hatching medium, one can routinely get oval-shaped prenauplii to emerge from their shell in double-membrane bags, a feature that others since have noted and have called "abnormal." However, even when NaHCO_3 is present, more recently, it has been shown that $10 \mu\text{M}$ cadmium will also cause double-membrane bags to emerge with a nauplius within, and apparently some nauplii eventually escape and become free swimming (Rafiee et al. 1986), contrary to our experience (although we have observed partially emerged nauplii struggling to swim with the double cuticular bag attached). The occurrence of these oval bags and the inability of nauplii to emerge from them are features useful in characterizing the water quality of hatching media. The rigid, double-membrane bag apparently can be produced under various circumstances, and thus, brine shrimp in postencystic development are useful as bioassay organisms in assessing water quality (Bag-

shaw et al. 1986, Trotman et al. 1987, Go et al. 1990, Trotman 1991).

In the process of clarifying postencystic development with regard to membrane and cuticle associations, we describe three modes of emergence of the nauplius, by TEM and SEM, and distinguish between the phenomena of emergence and hatching.

The most widely recognized mode of emergence is when the prenauplius leaves the cyst shell in a transparent, tapered bag (Myint 1956, Nakanishi et al. 1962, Wheeler et al. 1979, Sorgeloos et al. 1986, Trotman et al. 1987) known as the "parachute," "umbrella," or hatching membrane, becoming a nauplius 1 (L1). Just how many membranes form this transparent bag has been a point of confusion. Sato (1967a, 1967b) showed that there can be one or two, whereas some anostracans clearly have only one enclosing membrane when they emerge from their shell (Belk 1987). However, in *A. franciscana*, the number depends on conditions during hatching. Our observations with electron microscopy suggest that when the prenauplius emerges from the cyst shell in a tapered bag narrowest at the caudal end, the bag cuticle is single and is the EC2. Furthermore, the ICM portion of the EC1 remains stuck to the EC2 and is left trailing behind, attached or unattached to the shell (Myint 1956, Sato 1967a, Wheeler et al. 1979). However, when the bag containing the prenauplius is not tapered but oval, with equal to nearly equal size poles defining a prenauplius head-tail axis, then both the ICM and the EC2 have surrounded the prenauplius during emergence. Nonetheless, when two cuticles appear to bag the nauplius, only one may occur at a given position, as determined by TEM (Figs. 32–34). When the ICM is fused to the EC2, or alone in certain areas over a nauplius after its emergence from the shell (Fig. 34), the shape of the bag is due largely to the physical properties of the ICM alone (Figs. 6 and 32–34), because it is the stiffer of the two cuticles. Without the ICM covering the EC2, primary electron beam penetration with SEM may allow observation of the nauplius surface within the bag (Go et al. 1990). That is, with a single cuticle (EC2), beam penetration occurs, but when the ICM and EC2 are fused (Fig. 6), beam penetration is lost (to that depth at the same acceleration voltage).

With regard to shape, Go et al. (1990) refer to the nauplius surrounded by the ICM and the EC2 as having an "abnormal shape," as in our Figure 6, a shape we refer to as oval and one not typical of most bagged nauplii. Trotman (1991) also noted the oval-shaped nauplius, and, in the section of his article called "abnormal early development," suggested that this particular bag shape is due to "retention of the inner cuticular membrane." Our TEM evidence supports that interpretation, first suggested by Sato (1967a, 1967b). Perhaps one can predict the number of membranes associated with bagged prenauplii depending on the shape of the bag and the fate of the prenauplii, from the following details. The ICM is a smooth membrane, uniform in thickness, stiff, and electron dense, so that although showing some plasticity during emergence, it has a limited capacity to bend and especially to fold should it be transported outside the cyst shell (Figs. 5, 16, 28, and 31). It may or may not break away from the rest of the EC2 when the prenauplius emerges (cf. Fig. 2 with Figs. 3 and 4 of Rosowski et al. 1995). The ICM also appears to stretch to a limited extent (shows some elasticity) when the bagged nauplius is released from the shell in hatching medium lacking NaHCO_3 (Fig. 6). Nauplii in such oval bags generally are unable to escape and thus to feed, so hatching is perhaps best interpreted as escape from the EC2, not just the shell. The EC2 (hatching), when unassociated with the ICM except at its caudal end, is highly wrinkled when processed

for SEM (Figs. 3–5) or for TEM (Figs. 30 and 31). Furthermore, the EC2 folds readily and compactly in contrast to the ICM, which bends but only with broad curves (cf. Figs. 3 and 5 and Figs. 27 and 28). Thus, when the only cuticle surrounding the nauplius is the EC2, the shape of the parachute is determined more by the tapered shape of the nauplius than by any physical properties of the EC2 itself. Therefore, the EC2 is tapered when the nauplius 1 first emerges within this bag (Fig. 8), and it will have attained more than two times its volume than when it was in the cyst shell (Trotman 1980). Although the thicknesses of the ICM and the EC2 are similar whether inside or outside the cyst shell, nauplii 1 in EC2 bags typically escape (hatch), whereas those additionally surrounded by the ICM usually do not (see also Sato 1967b). The EC2 thus appears weaker than the EC1 and readily tears, allowing for escape of the nauplius 1, an interpretation supported by its less dense (more porous) image by TEM (Figs. 24, 25, 30, and 31). It should be noted that Sato (1967b) has provided evidence for a hatching enzyme at the head of the nauplius, which when activated, dissolves the EC2 only at the head region. Further study incorporating electron microscopy during these events would be useful in substantiating that report. Sato (1967a) also states that when the shell first cracks open (E1 stage of Nakanishi et al. 1962), two membranes are present "but cannot be seen clearly. . . ." Presumably, these are the same two membranes noted by Myint (1956) when prenauplii were prematurely forced from their shells. We interpret those two membranes as being the ICM and EC2 (Fig. 28). It should be noted that in an aborted hatching, when a nauplius starts to emerge but fails to leave the shell, typically both cuticles break at the same time and curl back toward the edge of the cracked shell (Fig. 7). The severe curling of the paired membranes suggests that at least the outer of the two, the ICM, is particularly elastic.

The EC1, a membrane reported to be highly impermeable by gastrulation to certain ions in order to allow for normal embryo development (De Chaffoy et al. 1978), consisted of the three major layers known previously from TEM (Morris and Afzelius 1967), plus numerous fracture surfaces within this membrane, presumably along the surface of fibrous lamellae, as now demonstrated by SEM (Figs. 11, 13, and 14). Because these lamellae are so variable in number, some may be artifactually produced. Given the smooth surface appearance by SEM of the ICM of the EC1 (when it is pulled away from the cyst shell, Fig. 5), we interpret the inner surface of the fractured cyst in Figure 17 as having its ICM missing, whereas the middle fibrous region and the OCM are still in place, with radial septa outlining the polygonal plates. By comparing the TEM and SEM images of the ICM, it became clear that septa occurred only in the outer third of the EC1 and just to the inside of the OCM (Figs. 20, and 27). The middle fibrous region of the tripartite EC1 lacks septa, and when the polygonal plates collapse in SEM specimen preparation, the septa remain in positive bas-relief on their outer surface (Fig. 17). It has been noted that the fibrous region is highly hydrophilic and "could act as a liquid reservoir and absorb part of the osmotic potential. As the fibrous region swells, the outer cuticular membrane may perform the dual functions of sealing the osmotic potential and mechanically preventing premature rupture of the fibrous region at the plate boundaries" (Trotman 1991). A recent study (Rosowski et al. 1995) showed that when a cyst is "deshelled," and the resultant embryo hydrated and then CPD, the EC1 surface is smooth, although slight primary electron beam penetration from SEM reveals the polygonal plates that lie to the inside of the OCM. Air drying of the same

material, however, clearly shows the rims of the polygonal plates (Fig. 2 in Rosowski et al. 1995) thus supporting the idea (Trotman 1991, see previous quote) that this region is highly hydrophilic.

Before the appearance in postencystic development of the EC2, we observed granular material in the subcuticular space between the ICM and the plasma membrane surface of the embryo. This material, within and outside the embryo, has been referred to as ribosome- or glycogen-like in morphology (Morris and Afzelius 1967, Rieder 1972). Granular material of the subcuticular space (= unlabeled region, Morris and Afzelius 1967; or "unassigned" region or layer, Trotman et al. 1980, Freeman 1989, respectively) is not only between the embryo and the ICM in early development, but we find it later between folds of the expanding embryo surface as the embryo becomes a prenauplius. Before the formation of the EC2, a similar fine, granular band occurs next to the inside of the plasma membrane opposite this extracellular space, and perhaps accumulates there before being secreted (Fig. 23). Fine granular material is then found after the EC2 has been produced, in which case it is sandwiched between the ICM and the EC2 but is greatly reduced in thickness (Figs. 25, and 30–33) compared with its earlier thickness outside the embryo (Fig. 22). This extracellular substance may serve as a lubricant in keeping the naupliar appendages apart when they are first forming and also may facilitate embryo expansion within the crowded spherical shell before the formation of the EC2. Once the EC2 is formed, the extracellular substance may then serve a second lubricating function, this time between the ICM and the EC2. In this capacity, during the only method of emergence that leads to hatching, the EC2 (enclosing the prenauplius) would slide past the inner surface of the ICM, leaving it within and attached to the shell. When in the parachute stage, the fine, granular matrix would be exposed to the surrounding medium and would be a likely substrate for bacteria. This material may be the glycerol reported within, and then outside the embryo on prenauplius emergence (Clegg 1964). The fine, granular particles that we illustrate here appear smaller than those within the cells illustrated by Morris and Afzelius (1967) and Rieder (1972), and described by them as glycogen-like in morphology.

The EC2, unlike the ICM, is fibrous rather than granular, is less electron dense, and has been described as being elastic and stretching before rupture (Belk 1987), which would explain why occasionally it is irregular in thickness (unlike the ICM). The EC2 remains adhered to the ICM during the embryonic development of the postmandibular segments within the cyst shell at the time of its cracking open. However, as paired naupliar appendages differentiate (the antennules, antennae, and mandibles), the EC2 presumably is produced over them as well, in regions well removed from the ICM (Fig. 27). At near the time of emergence of the prenauplius, but while the prenauplius is still within the shell, apparently, the EC2 is released from the surface of these paired appendages (Fig. 29), creating the loose parachute that must be inflated for the prenauplius to emerge from the shell. The initial inflation occurs at a stage in which the EC3 is incomplete (Fig. 29, appearing as a discontinuous embryo surface line), but with its final thickness established as the EC3 line-like segments form. The exoskeleton matures sometime between when the prenauplius is released from the shell and before it has hatched from the EC2 (Figs. 32–34). This maturation of the EC3 during prenauplius emergence was described by Freeman (1989) as the cuticle having "achieved a more defined structure." He noted in electron micrographs of other workers that the cuticle appeared to consist of an epicuticle (probably tanned) and an inner procuticle that was fibrous but not

layered. This description would fit our data on the EC3 as well (Figs. 32–34). The EC3 is thinner and unlayered in the procuticle region, unlike the EC3 of adults of *Artemia* (Freeman 1989) and of other genera of crustaceans (Schultz and Kennedy 1977, Stevenson 1985). The exoskeleton or naupliar cuticle (EC3) becomes recognizable in early development, before emergence, as disconnected dark lines (Figs. 28–31). The mature surface of the emerged nauplius I is always continuous in transverse view (Figs. 32–34).

In summary of cyst ultrastructure as it relates to nauplii emergence and hatching and the membrane configurations associated with those events, the following generalizations can be made. Because the shell is not required for nauplii emergence and hatching (Belk 1987, Spotte and Anderson 1988, Rosowski et al. 1995), it follows that its primary function is one of protection of the embryo and the tripartite EC1 that surrounds it at the time it is released by the female (Belk 1970). Although it has been assumed that the shell is chemically removed by deshelling treatments (Fig. 8 in Morris and Afzelius 1967), we find that the most of the tertiary base, about 0.3 μm thick (Fig. 35), may remain on the outer surface of the OCM after the deshelling treatment (cf. Fig. 36a and b). Further work is needed to determine the conditions under which a deshelling solution may yield cysts free of the TB, and if total removal of the TB is possible, if such TB-free cysts will also hatch.

After cyst rehydration, the EC2 is produced by the embryo in postcystic development. However, before this event, material of a fine, granular nature is secreted over the embryo epidermal cell plasma membranes, creating a region previously referred to as "undefined" or "unnamed." Later, similar fine granules appear between the ICM and the EC2, when the EC2 is complete and external to the EC3. The EC3 (nauplius I exoskeleton) surface is incomplete (discontinuous in transverse sections) until the prenauplius is out of the shell, or at least before the escape of the nauplius I from the EC2. The fine granular extracellular material becomes exposed to the hatching medium with the breaking of the ICM while inside the shell, or when the prenauplius has left the shell on emergence surrounded by the EC2. As others have suggested (see review by Trotman 1991), glycerol may be the osmoticum creating the turgor pressure that contributes to the breaking of the ICM. Perhaps this occurs in concert with the activation of a hatching enzyme in the head region of the prenauplius that is specific for the ICM, as presented by Sato (1967b) in an elegant experiment.

Trotman (1991) stated that "There is no compelling evidence that the point of rupture of the cyst wall is other than random in relation to the orientation of the embryo, as expected from the failure of a pressurized sphere." However, there is evidence for a specificity of orientation of the emergence crack in the shell, and

in attached shells it is with respect to the embryo axis which is parallel with the vector of gravity before naupliar emergence (Gouthro and Rosowski 1994). That is, the shell crack nearly always occurs in a 180° arc over the top of the prenauplius head, revealing the simple eye in an upward position, or in this same location in the case of cysts treated with a deshelling solution (Gouthro and Rosowski 1994, Rosowski et al. 1995). We do not know if the embryo rotates to bring about this parallel orientation of the embryo axis with the vector of gravity, or if the embryo differentiates along that vector so that rotation becomes unnecessary before emergence. Unlike the EC2, which is easily torn, the EC1 initially breaks inwardly in a straight line only to the position of its ICM at the time the shell crack first appears (Fig. 28; see also Fig. 6 in Trotman 1991). The prenauplius is thus surrounded by two membranes (ICM + EC2) as it emerges through the crack (Fig. 28; Sato 1967a, Sato 1967b). For the nauplius of a shelled cyst to become free swimming, osmotic forces must be sufficient to break the ICM as the prenauplius emerges from the shell in its EC2, and the EC2 must clear the shell completely without being broken. The cyst shell never breaks up during emergence because it is an elastic structure that is able to crack and flex open 180° during prenauplius emergence; because of this elasticity, however, it then closes behind the bag once the parachute with prenauplius has left (Fig. 5). If both membranes should break as the prenauplius begins to emerge (Fig. 7), osmotic pressure is lost and the animal becomes pinched by the shell. Without the capacity for the development of osmotic forces to inflate the EC2 bag, which would free it from the shell, it usually dies. If the nauplius leaves the shell completely but in a double cuticular bag (oval rather than tapered), the outer cuticle is so strong that usually by then no forces can be generated within the double bag to break the ICM and the nauplius remains trapped and starves. For cysts treated for the removal of their shell and then hatched (Rosowski et al. 1995), the EC1 must also break from the outside inward through its ICM in order for the release of the nauplius bagged within the EC2. Hatching in shelled or deshelled cysts is therefore dependent on forces leading to bag expansion, and these forces must be generated quickly and at a critical time for the ICM to break and release the EC2-bagged nauplius from the shell, or from the ICM and TB in "deshelled cysts." If the ICM is unbroken, hatching cannot occur. Because having a shell is not necessary for hatching, we believe that hatching should be defined as when the nauplius I escapes from the EC2 and becomes free swimming. Although the nauplius may appear to have hatched when it has emerged halfway out of the shell, if its outer two cuticles are broken, it generally cannot become free swimming and dies.

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ABSTRACTS OF TECHNICAL PAPERS

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CAN WE HAVE OUR OYSTER AND EAT IT TOO? A CASE FOR AQUACULTURE PARKS USING NON-NATIVE SPECIES. S. K. Allen, Jr.* and X. Guo, Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

The use of non-native species for the restoration of shellfish populations in the mid-Atlantic has received considerable debate. Arguments for their introduction include their potential ecological value as grazers on primary productivity and potential value as an aquaculture product. Arguments against them include their potential ecological harm if they become naturalized and their likely uselessness as a fishery product. We have been investigating the use of triploids as a population control measure for non-natives, primarily in the context of research experiments. Triploids produced by manipulation of meiosis of normal diploid oocytes immediately following fertilization are primarily, but not completely, triploid. Diploids, then, have to be eliminated by screening each and every individual. As a result, limited number of "safe" triploids can be deployed in the field. So-called "certified" triploids also seem to have another major problem: instability of chromosome content. Recently, we developed tetraploid oysters, where crosses of them to normal diploids yield all-triploids. Since each and every one (at least within the limits of our sampling power) is triploid, the population as a whole is sterile. There is no evidence of diploid cells resulting from instability of chromosome content in all-triploids. Theoretically, unlimited numbers of all-triploids could be produced to keep experimenters busy. But would it not be possible also to run unlimitedly large experiments, including research "parks" of all-triploid non-natives? Such large scale "experiments" have the virtue of remaining reversible provided that all-triploids are from disease free stocks and that all triploids remain all-triploid.

COOPERATIVE REGIONAL SELECTIVE BREEDING (CROSBREED) PROJECT: ODRP REPORT II. S. K. Allen, Jr.,* G. A. DeBrosse, S. E. Ford, and X. Guo, Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349; P. M. Gaffney, College of Marine Studies, University of Delaware, Lewes, DE 19958; D. W. Meritt, Horn Point Environmental Laboratory, Cambridge, MD 21613; K. T. Paynter, University of Maryland, College Park, MD 20742; E. M. Bureson and M. W. Luckenback, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The overall goal of the CROSBreed Project is (i) to complete two additional generations of selection using common breeding criteria across three mid-Atlantic sites and (ii) ultimately, make strains of oysters available to the industry or repletion programs as needed. A second generation of Dermo-selected synthetic lines were created in the summer of 1995 using the first generation Delaware Bay synthetic lines as the parent population. Following the larval culture period, competent eyed larvae were set cultchlessly using epinephrine and the spat reared in hatchery downwellers until they reach about 1 mm in shell length. At 1 mm, spat

were transferred to our upweller raceway system. During late August of 1995, at least 4,000 spat (to be deployed as replicates of 1,000) of each of the five HSRL groups, and the project control group were sent to each of the three sites: Delaware Bay, upper, and lower Chesapeake Bay. Each site also deployed a similar number of spat of a locally produced control group. Initial disease sampling for Dermo was conducted later that fall and only light infections were seen in 1995. Mean shell height in project groups sent from Rutgers Cape Shore Lab ranged between 13.5 and 15.2 mm. Neither the Delaware Bay site nor the lower Chesapeake site showed any appreciable mortality during the first three months of field deployment. The mortality at the upper Chesapeake site was substantial, ranging from 5–31%. Disease and size sampling have not been accomplished throughout the Spring and Summer of 1996 and data from these will be presented. In general, survival has been high in the face of low disease pressure (high rainfall on the east coast).

DEVELOPMENT OF NUCLEAR DNA MARKERS AND PEDIGREED FAMILIES FOR DISEASE RESISTANCE AND GENETIC MAPPING IN THE EASTERN OYSTER: PROGRESS REPORT. P. M. Gaffney, College of Marine Studies, University of Delaware, Lewes, DE 19958; S. K. Allen, Jr.,* Rutgers University, Haskin Shellfish Research Laboratory, Port Norris, NJ 08349; J. Pierce, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104.

We are developing a set of tools for the genetic analysis of *Crassostrea virginica* with the ultimate goal of locating genes affecting important traits such as disease resistance, growth, and survival. Genetic markers associated with quantitative trait loci can be used to enhance selective breeding programs, particularly in species such as oysters in which the time required to manifest the phenotype of interest may be several years. Our project consists of three components. First, we have used the bacteriophage P1 cloning system to construct a high-quality, high molecular weight *C. virginica* genomic library. The library contains approximately 10,500 individual clones, with an average insert size of ≈ 85 kb. The library is organized as 50 primary pools, each of which contains an average of 200 individual clones. These primary pools are stored as frozen glycerol stock cultures. The second component is an archive of oyster families (parents and progeny). Twenty-one single-pair matings of *C. virginica* have been made; parents, eyed larvae, and spat from all families have been archived. In addition, juveniles from 14 of the 21 families are currently being reared. These families will be used to make F_2 crosses, to provide a two generation pedigreed oyster archive. The third component is the development of additional nuclear DNA markers to supplement those currently available. We are using the P1 library to develop three types of markers: 1) microsatellites, 2) unique DNA sequences flanked by simple sequence repeats, and 3) random anonymous nuclear loci.

TRAGEDY OF THE COMMONS: "PRIVATE" VS. "PUBLIC" SHELLFISH HARVESTING. W. D. Anderson,* W. J. Keith, J. M. Monck, G. M. Yianopoulos, and R. W. Haggerty, Office of Fisheries Management, S.C. Department of Natural Resources, P.O. Box 12559, Charleston, SC 29422.

South Carolina's marine fisheries laws were modified in 1986 to provide greater commercial harvest opportunities for individuals without access to shellfish leases. Additionally, the State's 1992 marine recreational fisheries stamp law promoted personal gathering in the public domain. Management reclassifications of privately cultivated shellfish grounds to provide more State maintained shellfish resources have resulted in contrasting production and resource status. The "tragedy of the commons" occurs on State shellfish grounds as increasing recreational and commercial pressure is placed on the public domain. Pollution closures indiscriminately decrease acreage available for both public and private harvesting, while engendering unsolicited "shellfish sanctuaries" and "mitigation banks." Management strategies are discussed that compare shellfish stock enhancement (an aquacultural endeavor) to natural propagation.

THE EAST COAST OF NORTH AMERICA STRATEGIC ASSESSMENT PROJECT. J. Arbour,* Senior Adviser, Priority Issues Section, Environmental Protection Branch Environment Canada, 45 Alderney Drive, Dartmouth, Nova Scotia, Canada B2Y 2N6; S. Brown, Strategic Environmental Assessment Division, Office of Ocean Resources Conservation and Assessment, 1305 East West Highway, SSMC4, 9th Floor, Silver Spring, MD 20910; A. Menon, Shellfish Section, Environmental Protection Branch, Environment Canada, 45 Alderney Drive, Dartmouth, Nova Scotia, Canada B2Y 2N6; G. Howell, Ecosystem Science, Environmental Conservation Branch, Environment Canada, 45 Alderney Drive, Dartmouth, Nova Scotia, Canada B2Y 2N6.

The East Coast of North America Strategic Assessment Project (ECNASAP) brought government, university, and private sector parties together from both Canada and the United States in a joint project that focused on a single issue, the contamination of shellfish production waters. Strategic Environmental Assessment was applied to this issue with a geographical focus on the Gulf of Maine. The objective was to develop tools and methods that would facilitate the development of strategies for responding to the issue at both the large ecosystem level (Gulf of Maine) and at the local ecosystem level (individual estuary). The result of the project has been the development of a Gulf of Maine Shellfish register, an estuary-specific strategy for responding to the shellfish issue (St. Croix/Passamaquoddy Bay) and a set of desktop computer-based tools for analyzing and presenting information on the issue of contamination of shellfish production waters. The benefits derived from these products include the networking and linkages among multiple agencies and jurisdictions, a Gulf-wide assessment of the shellfish contamination issue, and a methodology for assessing the issue and developing strategic responses. This paper provides a

summary of the project, how it developed, and the products that have been produced.

RESTORATION OF OILED MUSSEL BEDS IN PRINCE WILLIAM SOUND, ALASKA, FIVE YEARS AFTER THE EXXON VALDEZ OIL SPILL. M. M. Babcock,* P. M. Harris, and S. D. Rice, NOAA National Marine Fisheries Service—Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AL 99801–8626.

The persistence of *Exxon Valdez* crude oil underlying some dense mussel (*Mytilus trossulus*) beds was measured 2–4 years post-spill. These beds were intentionally left untreated during cleanup activities, 1989–1991, because they provided physical stabilization and mussels were a food source for higher consumers. In 1992 and 1993, we documented 31 beds in Prince William Sound with sediment concentrations in excess of 10,000 $\mu\text{g/g}$ total petroleum hydrocarbons. Mussel concentrations of polynuclear aromatic hydrocarbons ranged to 10.0 $\mu\text{g/g}$. Samples from these beds contained the highest oil concentrations seen since 1990, the year following the *Exxon Valdez* disaster. Beds were not recovering rapidly naturally and oil from these mussels could be incorporated into the food chain. Our purpose was to evaluate a simple, manual technique for removing oil that was in close contact with overlying mussels. In 1994, cooperatively with the Alaska Department of Environmental Conservation and residents from Chenega, twelve mussel beds were restored. Mussels were removed and allowed to wash one tidal cycle on absorbent pads. Oiled sediment underlying the mussels was removed, uncontaminated sediment substituted, and mussels replaced. Bed stability and hydrocarbon concentrations were measured periodically. Short-term evaluation of the process indicated successful removal of oil from close contact with overlying mussels. By August, 1995, sediments underlying the restored bed showed an average decrease in total petroleum hydrocarbons of 98%. Our results show that this simple method, although labor-intensive, is successful by providing a buffer of uncontaminated sediment underneath mussels, thereby reducing the body burden of petroleum hydrocarbons in these mussels.

THE USE OF REGULATORY ENFORCEMENT TO ABATE THE POLLUTION OF SHELLFISH HABITAT. T. Bandrowicz* and E. Hall, U.S. Environmental Protection Agency, John F. Kennedy Federal Building, Boston, MA 02203.

A case study is presented on the use of enforcement to compel the abatement of shellfish habitat contamination in a small unsewered coastal community in Massachusetts. Decades of failing septic system problems in the community and the resulting direct and indirect discharge of bacterial pollution into the local estuary caused the restriction of historically productive shellfish beds. Although the community had several remedial plans prepared over a thirty year period, it did not implement any of their recommendations and the pollution problem persisted. It was only after the state and federal government threatened enforcement action that the

parties were able to reach agreement on an innovative solution that was then incorporated in a state court sanctioned consent agreement. In accordance with the order, the community is currently undertaking a multi-phased program of inspecting and identifying failing septic systems and other sources of contamination, and then selecting and implementing non-centralized remedies, including individual upgrades, communal systems, and innovative technologies. Issues to be discussed in the context of the case study include: the use of enforcement as a tool for directing remediation when various conflicting interests prevent effective solutions; some of the enforcement tools available under local, state, and federal law, including the innovative use of certain provisions of the federal Clean Water Act; examples of remedies that can be sought in an enforcement action to abate septic system pollution, in particular the decentralized approach taken in the case study; and the simultaneous use of incentives, such as state or federal grants.

POTENTIAL CONTRIBUTION OF THE AQUACULTURE INDUSTRY TO SHELLFISH STOCK ENHANCEMENT PROGRAMS. R. E. Bohn,* National Aquaculture Association, 111 W. Washington Street, Suite 1, Charles Town, WV 25414–1529.

The aquaculture industry can make a significant contribution to public shellfish restoration programs, and already performs this task for many private landowners. Much of the federal effort termed "aquaculture support" in the Departments of Interior and Commerce concerns similar activities in the restoration and enhancement of marine and freshwater fish stocks. While private shellfish aquaculture is typically viewed as high-density, physically-protected farming, most early efforts in the field resembled current proposed programs for depleted stock replenishment or restoration. Restraints on available growing areas, permit restrictions, and the threat of theft often drive aquaculturists to high-density production in marine and tidal waters. Much background knowledge of cost-effective methods with reasonable success rates have been developed by the industry, and examples will be given. Many of the potential scientific questions, many raised by fish enhancement programs such as salmonid stock enhancement, have been examined by aquaculturists. Issues of inbreeding depression and growth effects, hatchery vs. wild broodstock, and planting effects on existing wild stocks have often been examined in detail. Potential biotechnology advancements with applications toward restoration programs are also usually developed and practiced by the industry. Use of the aquaculture industry as a supplier of seed stocks is also likely to be found far more cost-effective than utilizing scarce state or federal resources. Greater knowledge and access to specialized techniques, equipment, and trained personnel make private industry more likely to produce better, and more products for restoration activities. Industry can also utilize Quality Assurance practices for consumer confidence when restoration efforts support recreational or commercial fisheries.

GAINING PUBLIC ACCEPTANCE OF AQUACULTURE AS A TOOL FOR SHELLFISH STOCK RESTORATION. C. Brown,* National Marine Fisheries Service, Office of Science and Technology, 1315 East-West Highway, Silver Spring, MD 20910.

Over-harvesting and poor water quality have resulted in a drastic decline in shellfish stocks available for harvest in the U.S. This, in turn, has led to a greater reliance on imported shellfish to meet consumers' demand for safe seafood. The role of the U.S. as a major importer of seafood has added about \$2.2 billion to the Nation's trade deficit. Aquaculture can and should play a significant role in restoring the nation's shellfish stocks and shifting the U.S. from an importer to a major exporter of seafood. Techniques for culturing molluscan bivalves are well known. Some traditional methods, however, may no longer be appropriate in many areas. Coastal property value is at a premium and the public is becoming increasingly protective of its coastal ecosystems. The public is demanding healthy coastal ecosystems and is wary of private enterprises that interfere with its access to the waterways. General acceptance of aquaculture as a tool for shellfish stock restoration can be obtained if the systems are environmentally sensitive. This means that culture and grow-out systems should be designed to minimize the public's concerns, both real and perceived. These concerns include: competition with public uses, diminished aesthetic appeal, reduction of habitat for natural stocks, introduction of non-native species, reduction of genetic diversity, and lowered water quality. Concomitantly, aquaculture endeavors should maximize their potential for reducing the trade deficit, creating job opportunities, and lessening fishing pressure on declining wild stocks.

LIFE CYCLE STUDIES OF *HAPLOSPORIDIUM NELSONI* (MSX) USING PCR TECHNOLOGY. E. M. Burreson,* N. A. Stokes, and B. S. Flores, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062; S. E. Ford and K. A. Alcox, Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

The oyster pathogen *Haplosporidium nelsoni*, the agent of MSX disease, has caused extensive oyster mortality in the eastern United States since 1957. Much has been learned in the past four decades; however, the complete life cycle of *H. nelsoni* remains unknown. Attempts to infect oysters directly with *H. nelsoni* spores have been unsuccessful, thus leading to speculation that parasite transmission between oysters occurs via an obligate intermediate host. We have developed a diagnostic assay using the polymerase chain reaction (PCR) which detects *H. nelsoni*-infected oysters with much greater sensitivity than traditional histological examination. This assay has been optimized for use with environmental samples and the *H. nelsoni*-specific PCR primers are being used in the search for the putative intermediate host(s). Weekly samples of water and sediment fractions and of macroin-

vertebrates have been taken from MSX-endemic areas of Delaware Bay and York River, VA since March 1996. Total genomic DNA has been extracted from each sample and subjected to PCR amplification. Some of the samples have yielded *H. nelsoni* PCR product and we are currently optimizing the protocols to conduct *in situ* hybridizations on these samples using the *H. nelsoni*-specific DNA probe. We are also continuing our sampling/PCR regime.

CHLORINE TOLERANCE OF *PERKINSUS MARINUS*. D. Bushek,* R. Holley, and M. Kelly. Baruch Marine Field Laboratory, University of South Carolina, P.O. Box 1630, Georgetown, SC 29442.

Perkinsus marinus causes extensive mortality in eastern oyster (*Crassostrea virginica*) populations annually and is therefore a major problem for oyster stock enhancement, management, and restoration. Human transport of oysters and subsequent disposal of infected tissues into estuarine and marine waters may spread virulent races. We explored chlorination as a method to kill *P. marinus* prior to disposal of *P. marinus*-contaminated materials. *In vitro* cultured parasites and infected oyster tissues were exposed to various dilutions of household bleach for 0.5, 4, and 18 hours. Neutral red viability assays indicated the addition of 300 ppm Cl_2 to filtered seawater (a 1:180 dilution of bleach) was required to kill *in vitro* cultured parasites within 0.5 hours. In culture medium, 400 ppm was required with an incubation time of 4 hours. Subsequent *in vitro* proliferation of parasites confirmed viability. These data indicate that standard bleach sterilization procedures, which use chlorine concentrations of 10–25 ppm, are ineffective against this pathogen. Alternatively, 1 hour exposure to fresh water or 1 hour incubation of cultured parasites in sea water or culture medium at 50°C effectively killed the parasites. Additionally, results from ongoing experiments using infected tissues will also be presented along with data on the parasite's tolerance to temperature and osmotic shock. Parasites embedded in tissues will likely require higher chlorine concentrations, higher temperatures, or longer incubation times due to protection from the surrounding tissue.

OYSTER REEFS AS STRUCTURAL AND FUNCTIONAL COMPONENTS OF TIDAL CREEKS: AN ONGOING ECOSYSTEM EXPERIMENT. R. F. Dame, E. Koepfler, L. Gregory, and T. Prins, Coastal Carolina University, Conway, SC 29526; **D. Allen, D. Bushek,* C. Corbett, D. Edwards, B. Kjerfve, A. Lewitus, and J. Schubauer-Berigan,** Baruch Marine Laboratory, University of South Carolina, Georgetown, SC 29442.

An ongoing replicated ecosystem level experiment is described that addresses the structural and functional role of oyster reefs in tidal creeks. A set of eight similar tidal creeks was standardized for oyster dry body biomass to creek water volume at bank full con-

ditions. During the first year, all creeks are being analyzed for structural aspects including reef and water column biomass and diversity, water chemistry, food web structure, as well as functional attributes including oyster growth, total creek metabolism, and nutrient cycling. After the initial year, four creeks will have their oyster reefs removed and the structure and function of the two classes of creeks will be compared for a year. In the final phase of the project, artificial dams of oyster shells, analogous to real oyster reef dams, will be placed in two creeks with and two creeks without oysters in order to simulate an increase in water mass residence time. The four classes of creeks will be followed as before. We believe that this replicated experimental design will further our understanding of the role of oyster reefs in sustaining their ecosystems.

ROUND TABLES, COMMUNITY STEWARDS AND SEPTIC SOCIALS. G. D. Caine,* Aquaculture and Commercial Fisheries Branch, Ministry of Agriculture, Fisheries and Food, 2500 Cliffe Avenue, Courtenay, British Columbia, Canada V9N 5M6.

Increasing urbanization and industrialization of British Columbia's (BC) coastal areas is resulting in more pollution events and degradation of shellfish growing water quality, affecting both the commercial and recreational shellfish industries. Baynes Sound, on the central east coast of Vancouver Island, is the hub of BC's shellfish industry, generating almost fifty percent of the total oyster production in the Province. The Baynes Sound Round Table (BSRT) was formed in August of 1994 in response to alarming increases in fecal coliform counts throughout the Sound, affecting not only the largest concentration of shellfish farms in BC, but also the local communities who view Baynes Sound as a jewel in the crown of Canada's recreation capital. It was obvious from the outset that the magnitude of this problem, and its myriad pollution sources, could not be addressed by one group or government agency alone. An organized, cooperative effort generated at the community level would have to be brought to bear if the pollution tide was to be turned. The Round Table concept for multi-party mediation is not new, but it is the first time in Canada that this model has been applied to a problem of such scale. The BSRT brings government regulators, industry stakeholders, local politicians, and environmental groups to a focus on shellfish growing water monitoring and remediation. Since its inception, the BSRT has launched a variety of programs to monitor and remediate poor water quality in the Sound, as well as sponsoring public education and informational meetings to heighten community awareness. From this have come a number of innovative, community-driven programs, such as the formation of the "Sound Stewardship" groups, volunteer "Hotspot" monitors, "Adopt-a-Stream" neighborhoods, and "Septic Socials," which teach local residents about their septic systems and how to maintain them. Government regulators cannot be expected to accomplish this alone. Efforts at the

grassroots level are the key to reversing the pollution sources threatening shellfish growing waters and habitat in BC.

GROUND-WATER TRANSPORT OF BACTERIA AND NUTRIENTS FROM SEPTIC DRAINFIELDS, ISLE OF PALMS AND JOHNS ISLAND, SOUTH CAROLINA. F. H. Chapelle,* J. E. Landmeyer, J. M. Barton, and P. M. Bradley, U.S. Geological Survey, 720 Gracern Road, Suite 129, Columbia, SC 29210.

Two septic tank drainfield sites, one on the Isle of Palms and one on Johns Island, South Carolina, were instrumented with wells and monitored for one year to investigate the transport of bacteria and nutrients in shallow ground-water systems. The data show that coliform bacteria, *E. coli* bacteria, and nitrate were delivered to shallow ground water at both sites. Concentrations of coliform bacteria varied from 10^1 to 10^5 cells per 100 mls of ground water in the aquifer below each drainfield over the one-year period. Concentrations of *E. coli* ranged from zero to 10^2 cells per 100 mls over the same time period. Nitrate concentrations at the septic outfalls ranged from 30 to 100 mg/L. Concentrations of *E. coli* and nitrate decreased below measurable levels less than ten feet downgradient of the septic outfall at the Johns Island site, and less than 50 feet downgradient at the Isle of Palms site. Concentrations of coliform bacteria, which are naturally present in these ground-water systems, also decreased to background levels (10^1 – 10^3 cells/100 mls) near the outfalls. These data suggest that while bacteria and nutrients are delivered to shallow ground water from septic systems, natural attenuation processes prevent significant transport of these contaminants to nearby surface-water bodies. This, in turn, suggests that properly spaced and correctly maintained septic systems are not likely to contaminate shellfish with nutrients or bacteria.

STRESS PROTEINS AND INDUCED TOLERANCE IN *CRASSOSTREA GIGAS*. J. S. Clegg, K. Uhlinger, S. Jackson, G. N. Cherr,* E. Rifkin, and C. S. Friedman, University of California—Davis, Bodega Marine Laboratory, P.O. Box 247, Bodega Bay, CA 94923.

The hypothesis under test is that induced tolerance to thermal stress may confer resistance, via stress proteins (SPs), to oyster pathogens and other forms of stress. Initial studies have characterized the heat shock response. Thermotolerance was induced (for up to two weeks) in Pacific oysters by heat shock at 37°C for 1 hr which enabled them to withstand an otherwise lethal temperature (43°C, 1 hr). Thermotolerance was accompanied by increases in two constitutive members of the SP-70 family (69 & 72 kD) in gill tissues and the induction of a new isoform (SP-67); the latter may be a general biomarker for stress in these oysters. Carbon 14-labeled amino acid mixtures were used to evaluate protein synthe-

sis in gill tissue *in vitro* from control and previously heat-shocked oysters (fractionation and SDS-PAGE/autoradiography). At present, there is a strong correlation between induced thermotolerance at the organismal level and the synthesis and levels of members of the SP-70 family; the relationship between heat shock, induced thermal tolerance, and hyposalinity is also under investigation. Studies are underway which differentiate pathogen and host stress protein expression in *Nocardia crassostreae*-infected oysters. Elevated levels of SP-70 were observed immediately after heat shock in *Nocardia*; SP-70 returned to control levels within 2 hr. The rapid return of bacterial SPs to control levels as compared to oysters (up to 14 days) suggests that bacterial SP-70 can be differentiated from host SP-70 by Western blot analyses at extended times after heat shock.

THE INTERACTION OF THE OYSTER PROTOZOAN PARASITE, *PERKINSUS MARINUS* AND ITS HOST, THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*: A PROGRESS REPORT. F. L. E. Chu* and A. K. Volety, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062–1346.

This paper reviews our investigations on the interactions between *Perkinsus marinus* and the eastern oyster. Our studies revealed that: 1) oysters at higher temperatures had higher concentrations of circulating hemocytes, percentage of granulocytes, and phagocytic capability, but did not result in fewer or less intense *P. marinus* infections; 2) plasma lysozyme concentrations were negatively correlated with temperature and salinity; 3) in both summer and winter months, oysters residing in a low salinity habitat had higher lysozyme concentrations in plasma and mantle tissues than oyster's inhabiting high salinity areas; 4) oyster hemocytes recognized and phagocytosed *P. marinus* but only limited degradation of the parasite occurred; 5) only a few individual oysters were capable of destroying the parasite intracellularly; 6) no chemiluminescence response was elicited from oyster hemocytes when challenged by *P. marinus*; and 7) *P. marinus* cells and extracellular products containing acid phosphatase suppressed the production of reactive oxygen intermediates from host hemocytes. While these results imply that the cellular mechanisms may not be effective in defense against *P. marinus*, the higher plasma lysozyme in oysters at low temperature and salinity may offer an unfavorable environment for the development of the parasite and/or further weakening of parasite activity. Our findings also suggest that the parasite possesses virulence factors such as acid phosphatase(s) (AP), through which the parasite effectively evades the host's defense mechanism. We are currently purifying lysozyme from the oyster and testing its effect on *P. marinus*' pathogenicity and viability. Future studies will concentrate on: 1) investigating the role of oyster lysozyme in host defense; 2) determining whether AP is responsible for the virulence/infectivity of the parasite; and 3) selecting, for breeding programs, individual oysters capable of destroying the parasite.

INTERTIDAL OYSTER REEF HABITAT ASSESSMENT AND RESTORATION: EVALUATING HABITAT USE, DEVELOPMENT AND FUNCTION. L. D. Coen,* E. L. Wenner, D. M. Knott, B. Stender, N. H. Hadley, M. Y. Bobo, and D. L. Richardson, Marine Resources Research Institute, S.C. Department of Natural Resources, Charleston, SC; M. A. Thompson and R. E. Giotta, University of Charleston, SC, Graduate Program in Marine Biology, 217 Fort Johnson Road, Charleston, SC 29412.

In 1994 we began a long-term study to evaluate whether intertidal oyster reefs play an important role in southeastern estuarine ecosystems. Ultimately, this information will be used to formulate strategies for the management of this habitat and development of habitat restoration and mitigation methods. In South Carolina, over 95% of the oysters grow intertidally (tidal range >2 m), versus subtidally, making them different from oyster habitats elsewhere. We utilized an experimental approach to construct replicate experimental reefs to follow habitat development (recruitment and succession) and use by "transient" and "resident" species. Two sites are being studied, each with three replicate experimental reefs of 23 m². We are also collecting environmental data (DO, salinity, pH, turbidity, intertidal and subtidal temperatures), monitoring oyster diseases (monthly Dermo and MSX) and other important life history parameters (SPF growth, spat set, reproduction) on experimental, as well as adjacent natural reefs. To date, we have collected over 34 species of fish and decapod crustaceans that make transient use of the reefs, with densities often exceeding 5,600 individuals/23 m² reef. As many as 56 resident macroinvertebrate species have been identified from seasonal reef samples, first taken after only 5 months post-construction. By month seven (May, 1995), large numbers of xanthid crab recruits (<1.5–3 mm cw) were observed on both natural and experimental reefs. By initiating and following the long-term reef development, we will be able to explore potential changes in reef habitat status and function during reef succession.

RE-INTRODUCTION OF OYSTER CULTIVATION IN THE SLUICE-DOCK IN OSTEND, BELGIUM. P. Coutteau,* N. Coolaet, and M. Caers, Laboratory of Aquaculture and Artemia Reference Center, University of Ghent, Ghent, Belgium; P. Bogaert, Flemish Institute for the Environment, Ostend, Belgium; R. De Clerck, Fisheries Research Station, Ostend, Belgium.

Belgian oysters (called "Ostendaises" during the "Belle Epoque" times) were renowned in the period 1870–1913 for their good flavor. At that time, over 2,000 tons of half-grown flat oysters were imported yearly from UK for on-growing in 26 Belgian oyster parks employing 275 people. In 1935 oyster cultivation starting from wild spat captured on limed tiles was introduced by stocking a population of native flat oysters *Ostrea edulis* in the Sluice-Dock, a shallow lagoon (1.5 m deep and covering an area of 86 ha) connected to the harbor of Ostend by sluices that are kept closed under normal conditions. This method allowed the collection of millions of natural spat each year until the early seventies, when the deterioration of the water quality due to pollution prevented the wild oysters from com-

pleting the natural reproductive cycle in the Sluice-Dock. As a result, oyster on-growing ceased completely in 1974. Due to the installation of a sewage treatment plant in Ostend and the sanitation of the Sluice-Dock through lime treatments in 1990–1991, water quality appears to have improved. A wild oyster population (mostly Pacific oysters *Crassostrea gigas*) has settled and grown to adult size over the last years and interest is growing to reactivate the traditional cultivation method by restoring the native oyster population. This paper reports on a preliminary survey of the suitability of the Sluice-Dock for oyster restoration, including a monitoring program for abiotic and biotic parameters and a growout trial with Pacific oyster seed (initial shell length of 2 cm) in various sites.

NON-MARKET BENEFITS OF SHELLFISH RESTORATION. J. Diamantides,* Economic Analysis Inc., 10 Overhill Road, Providence, RI 02906–3718.

Restoration of shellfisheries provides significant economic benefits to local communities in terms of both market and non-market values. Possibly the largest economic benefit generated by improved shellfisheries is the non-market value to recreational shellfishers. Recreational shellfishers value their shellfishing experience by considerably more than the price, in terms of license fees if any, they pay to enjoy the activity. They derive value from their shellfishing experience through their harvest, through the act of shellfishing itself, being out on the water and engaging in an act of self sufficiency, and through identifying with a community that remains tied to the natural resources of the area. Although non-market values are routinely estimated for other marine recreational activities, there has been very little work in the estimation of non-market values of recreational shellfishing. This research effort is intended to assist local communities in the valuation of their recreational shell fishery, including estimating the value of opening currently closed areas. The first stage of the research estimates the non-market values of recreational shellfisheries at sample locations along the New England coast using random utility models. In the second stage of the research, a benefit transfer model is constructed that provides communities not included in the research sample the opportunity to evaluate the non-market benefits of their recreational shellfisheries. The result of this research effort is a tool that communities can use to quantify the economic benefits of restoring their recreational shellfisheries when assessing the merits of potential water quality initiatives in a benefit-cost analysis.

STOCK ASSESSMENT OF THE BLUE MUSSEL *MYTILUS EDULIS* L. IN LIMFJORDEN, DENMARK 1993–1995 AND THE ECOLOGICAL CONSEQUENCES OF MUSSEL FISHERY. P. E. Dolmer,* E. Hoffmann, and P. S. Kristensen, Danish Institute for Fisheries Research, Department of Marine Fisheries, Charlottenlund Castle, DK-2920, Charlottenlund, Denmark.

In Limfjorden, a 1575 km² brackish enclosure in the northern part of Denmark, an extensive fishery of natural stocks of blue mussels *Mytilus edulis* takes place with annual landings exceeding 110,000 tonnes of mussels. The intensive mussel fishery is con-

flicting with recreation and conservation. It is believed that the mussel fishery removes mussels important for the control of the phytoplankton biomass, increasing the frequency of periods with oxygen depletion. In April 1993, 1994, and 1995 the standing stocks of blue mussels were estimated in 16 subareas in Limfjorden. In addition, the mussel fishermen reported the catch weight of mussels from each subarea to the Ministry of Agriculture and Fisheries and by dividing the reported fishery of mussels by the estimated stock size the exploitation rate of the mussel stock in each subarea was calculated. In 1993–1994, a significant correlation between the change in stock size in each subarea and the exploitation of the stocks was observed indicating that the mussel fishery reduced the standing stock of mussels. In 1994 to 1995 no correlation was observed between the exploitation of mussel stocks and the fraction of the standing stocks landed. In summer 1994 a period of oxygen depletion was observed in some parts of Limfjorden. In subareas with oxygen depletion a massive reduction of the mussel stock was observed, whereas a significant increase was observed in subareas with an oxic condition. The mass mortality results in release of nutrients and reduced the filtration capacity of the mussel stock.

PERKINSUS MARINUS: IMMUNOASSAY DETECTION IN OYSTER TISSUES AND ENVIRONMENTAL SAMPLES AND IN VITRO EXPERIMENTAL SYSTEMS. C. F. Dungan.* Maryland Department of Natural Resources, Cooperative Oxford Laboratory, 904 S. Morris Street, Oxford, MD 21654 (and listed collaborators).

The first antibodies for detection of *Perkinsus marinus* were developed and their binding specificities described (Dungan & Roberson 1993, *Dis. Aquat. Org.* 15:9–22). Antibodies to *P. marinus* were used to develop a flow cytometric immunoassay which provided the first method for enumerating pathogen cells dispersed in estuarine waters (Roberson & Dungan), and this immunoassay was used to test longstanding hypotheses about the dynamics and mechanisms of dermo disease transmission in Chesapeake Bay (Burreson, Dungan & Roberson). Histological immunoassays were used to localize *P. marinus* portals of pathogen entry in uninfected oysters challenged in both the laboratory and the field (Burreson & Dungan). Antibodies were freely distributed to, and used by, several other oyster disease research investigations. A tetrazolium-based cell proliferation assay was validated for use with *in vitro* *P. marinus* isolates, and was used to optimize culture conditions and screen for drug sensitivities (Dungan & Hamilton 1996, *J. Eukaryot. Microbiol.* 42:379–388). This colorimetric cell proliferation assay was used in tandem with a flow cytometric viability assay to screen potential chemotherapeutants for activity against *P. marinus* (Roberson & Dungan). The assay was also used to document inhibitory effects of a recombinant antimicrobial peptide under consideration as a transgenic insert for oyster disease resistance enhancement (Pierce, Malloy, Salvador & Dungan submitted, *Mol. Mar. Biol. Biotechnol.*). One *P. marinus* isolate was

deposited at the American Type Culture Collection for unrestricted distribution to requesting researchers, and several isolates were directly distributed to other oyster disease research programs.

SHELLFISH CLOSURE RESPONSE STRATEGIES IN PUGET SOUND. D. C. Fagergren,* Puget Sound Water Quality Action Team, Office of the Governor, P.O. Box 40900, Olympia, WA 98504–0900.

The Puget Sound Water Quality Management Plan lays a broad framework for protecting water quality and estuarine resources in the Puget Sound basin of Washington state. Amendments to the Puget Sound Plan in 1991 added requirements for targeted and immediate response to shellfish downgrades. Since then, "shellfish closure response strategies" have been developed and implemented in six areas around the Sound. Complementing these requirements, the Washington State Legislature passed legislation in 1992 expanding authority for county governments to voluntarily establish "shellfish protection districts" to better control nonpoint source pollution in shellfish watersheds. The legislation also included a provision requiring counties to create shellfish protection districts in response to growing area downgrades. Seven districts have been created thus far, five of which were created as a result of shellfish downgrades. The closure response strategies and shellfish protection districts have achieved mixed results, reflecting variability in growing area conditions, land use practices, pollution sources, citizen commitment, local government capacity, political will, and state financial and technical support. Although water quality improvements have been achieved, none of the targeted closure areas has yet been upgraded. Downgrades will likely continue in the region because of enhanced monitoring, population growth, expanded classification of recreational beaches, and other factors. The closure response process must be streamlined and strengthened to achieve better results and to effectively complement other preventive strategies. Citizens and local governments, while already key players in this process, will undoubtedly play increasing significant roles in shellfish restoration efforts.

A PROMISING CHEMOTHERAPY FOR PERKINSUS MARINUS-INFECTED OYSTERS. M. Faisal,* J. F. La Peyre, and S. L. Kaattari, School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Chemicals known for their antimycotic and antiprotozoal properties have been screened for their efficacy against *Perkinsus marinus*. Unfortunately, none of the drugs studied have proven totally effective in treating or preventing *P. marinus*. The inability to cure infected oysters could be attributed to either the protozoan's resistance or the inability to deliver the chemotherapeutant into oyster tissues in effective concentrations. Bacitracin has been used in the chemotherapy of bacterial infections in man and animals. The sensitivity of *P. marinus* to bacitracin was investigated. The *in vitro* growth rates of two isolates of *P. marinus* were significantly reduced by bacitracin. The sensitivity of *P. marinus* to bacitracin

was further confirmed *in vivo*. In the first experiment, each oyster was injected with 10^7 *Perkinsus*-1 cells, then fed bacitracin encapsulated in lipid vesicles daily for six weeks. Parasite body burden was significantly reduced in oysters administered 5 mg/mL ($3.3 \times 10^4 \pm 2.5 \times 10^4$ hyphospores/g wet tissue) as compared to control oysters ($3.2 \times 10^5 \pm 4.7 \times 10^5$ hyphospores/g) that received encapsulated sea water only. In the second experiment, naturally infected oysters (average $13 \times 10^6 \pm 31.7 \times 10^6$ hyphospores/g) received encapsulated 10 mg/mL bacitracin for 10 weeks. Treated oysters had significant lower levels of infection ($3.61 \times 10^6 \pm 6.2 \times 10^6$ hyphospores/g) than control oysters ($77.42 \times 10^6 \pm 150.63 \times 10^6$ hyphospores/g). We find that bacitracin clearly has promising potential for the use in *P. marinus* chemotherapy.

JUVENILE OYSTER DISEASE PROGRESS REPORT: RESISTANCE STUDIES, 1996. C. A. Farley* and E. J. Lewis, National Marine Fisheries Service, Cooperative Oxford Laboratory, 904 S. Morris St., Oxford, MD 21654; D. Relyea and J. Zahltla, Frank M. Flower Co., Oyster Bay, NY 11771; G. Rivara, Cornell University, Cooperative Extension, Southold, NY 11971.

This is the third year of juvenile oyster disease resistance studies in *Crassostrea virginica*. The first year showed up to 7 times better survival of progeny of a brood stock selected on the basis of (1) survival, and (2) presence of characteristic shell checks. The second year F_1 and F_2 progeny were evaluated against progeny from susceptible brood stocks deployed in seven different sites. Survival of F_1 and F_2 resistant seed was 7 to 25 times better than the susceptible seed. In 1996, we developed F_2 and F_3 generations and a comparable-aged susceptible control population which were deployed in five different sites. Early results after five weeks of exposure demonstrated mortalities of 24–46% in the susceptible population compared with 2–16% for the two resistant populations. No significant differences were seen between the two resistant populations. Survival was 6 to 7 times better in the resistant populations which has resulted in a successful management strategy for avoiding the devastating effects of this disease.

POLLUTION ABATEMENT IN SHELLFISH WATERS: A SUCCESS STORY. M. J. Garreis,* K. Brohawn, G. Keller, T. Yu, J. Kurman, and C. Holland, Maryland Department of the Environment, 2500 Broening Highway, Baltimore, MD 21224.

In 1968, the MDE embarked on an ambitious program to re-open major shellfish harvesting areas. The program strategy focused on controlling sewage discharges and used a combination of treatment plant upgrading, intervention strategies, waste management planning, and financial incentives to improve quality of effluents and to eliminate bypassing at treatment plants and pumping stations. Implementation stretched over 20 years and involved existing and new sewage treatment plants. The program resulted in a decrease from 27% (320,655 acres) in 1970 to 5% (58,562 acres) in 1987 in areas restricted to shellfish harvesting. Emergency re-

strictions on shellfish waters decreased from approximately ten to fifteen per year in 1974 to about one every 5 years in 1990. In 1995, a reevaluation of the program strategy was made to determine if additional controls were necessary, or if existing controls should be maintained. This paper summarizes the program and the results of the reevaluation.

PCR DETECTION OF THE BACTERIAL PATHOGEN IN OYSTER NOCARDIOSIS. A. Gee* and R. A. Elston, Biology Department, Pacific Lutheran University, 12182 South 121st Street, Tacoma, WA.

Pacific oyster nocardiosis (PON) is a widespread husbandry disease of adult *Crassostrea gigas* that periodically causes significant mortality, particularly during warm temperature periods. (Elston et al. 1987, Friedman et al. 1991). The bacterial pathogen is a Gram-positive, mycolic acid producer of the genus *Nocardia*. Friedman gave us six strains of *Nocardia* isolated from diseased animals in Washington state and in British Columbia for our current research: a study to identify the isolates by 16S rDNA sequence comparisons and to use sequence information to construct primers for PCR detection of PON pathogens. Sequence comparisons of 16S rDNA indicates that all six isolates are identical and are closely related to *N. seriolae*. This observation is consistent with Friedman's earlier hypothesis based on a very thorough study of morphological traits, mycolic acid profiles, and biochemical properties (Friedman, personal communication). Thus, detection tools based on the sequence information of any isolate should work with all known isolates of PON. We have two sets of PCR primers and one oligonucleotide probe for detecting PON bacteria. One primer set amplifies a portion of the 16S rDNA template from more than one genus of mycolic acid producing bacteria, including *Nocardia*. Nested in this first set is a primer set that is specific for the genus *Nocardia*. The molecular probe is a pathogen specific sequence within the *Nocardia* specific amplicon. Primer sets and the probe can be used alone or in combination dependent on the requirements for sensitivity and specificity.

NANWALEK, PORT GRAHAM, AND TATITLEK SUBSISTENCE CLAM RESTORATION. J. J. Hetrick,* P. B. Schwalenberg, and D. Daisy, Exxon Valdez Oil Spill Restoration Project 95131, Chugach Regional Resource Commission, 4201 Tudor Centre Drive, Suite 211, Anchorage, AL 99518.

Clams were once a major subsistence resource in the Native communities of Nanwalek and Port Graham in lower Cook Inlet and Tatitlek in Prince William Sound. Local clam populations have been decreasing in recent years and their contribution to the subsistence harvest has been greatly reduced. There are probably several reasons for this including changes in current and beach patterns, increasingly heavy sea otter predation and the Exxon Valdez oil spill. The oil spill impacted the wild clam populations and their importance as a subsistence food in two ways. First, some clam beds suffered from direct oiling. Second, even though many

clams were not directly impacted by the oil, they have a tendency to accumulate, concentrate, and store the toxic contaminants from non-lethal amounts of oil. This has badly eroded the confidence of the villagers in the helpfulness of the remaining wild clam populations as a subsistence food. This project will attempt to restore local shellfish populations for subsistence use.

CRASSOSTREA VIRGINICA PATHOGENS IN CHESAPEAKE BAY OYSTER POPULATIONS: A DUAL DISEASE SIMULATION MODEL OF PARASITE-HOST INTERACTIONS OVER A LARGE SPATIAL SCALE. E. E. Hofmann,* and J. M. Klinck, CCPO, Crittenton Hall, Old Dominion University, Norfolk, VA 93529; E. N. Powell and S. Ford, Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349; S. Jordan, Cooperative Oxford Laboratory, 904 S. Morris St., Oxford, MD 91654.

The objective of this research project is to develop, validate, and implement a mathematical model of the effects of the two lethal oyster parasites, *Perkinsus marinus* and *Haplosporidium nelsoni*, on populations of the eastern oyster, *Crassostrea virginica*. Initial efforts have focused on developing a model for *H. nelsoni* which is capable of reproducing the observed seasonal cycle of prevalence and intensity of MSX in oyster populations. The oyster-MSX model includes processes that govern the growth and death of *H. nelsoni* in the epithelial and systemic oyster tissue. These processes are mediated by ambient environmental conditions of temperature, salinity, food availability, and turbidity, as well as by density-dependent biological controls. Simulations that use environmental conditions characteristic of Delaware Bay accurately reproduce the observed increase in MSX prevalence and intensity in summer and fall, the decrease in winter, and increase in the following spring. These simulations show that the amount of cold exposure (degree days) in the winter, the onset of the spring bloom, and the summer salinity are the primary factors controlling MSX prevalence and intensity. Additional simulations using environmental conditions from the Chesapeake Bay are able to reproduce the observed along-Bay gradient in MSX prevalence and intensity. The interaction of *H. nelsoni* with a size-structured oyster population is also explored in a series of simulations. These simulations illustrate the effect of events such as MSX sporulation on altering the prevalence and intensity of this disease in different sized oysters.

FIFTY-SIX YEARS OF OYSTER HABITAT RESTORATION AND POPULATION ENHANCEMENT IN MARYLAND. M. L. Homer,* Maryland Department of Natural Resources, Piney Point Aquaculture Center, P.O. Box 150, Piney Point, MD 20674.

Since 1940, Maryland has funded a large-scale oyster bar restoration and enhancement program. During the last 50+ years, an average of 5 million bushels of fresh and dredged shells have been planted on Maryland's oyster bars each year. Over the same pe-

riod, 625 thousand bushels of seed oysters (about 500 million oysters) have been transplanted annually. The planted shell totals exceed harvest removal by a factor of 2, while the seed oyster transplantation figures represent a one for one replacement of harvested oysters. Based on current expenses, this program costs about \$3 million per year. Between 1940 and 1982, Maryland's oyster harvest averaged nearly 3 million bushels each season. Since 1982, harvest yields have averaged 0.65 million bushels, and since 1987, 0.25 million bushels. Between 1940 and 1975, it has been estimated that Maryland's oyster bars have decreased in acreage by about 30%, losing another 15–20% since 1975. A reasonable question to ask is are current restoration efforts too limited to be effective or are other factors driving the decline of harvested oysters and the loss of habitat. The answer is contained within a simple characterization of Maryland's oyster populations. Throughout the range of the eastern oyster, the lowest recruitment generally and historically occurs in Maryland's Chesapeake Bay. How could this area have produced more oysters than any other East Coast or Gulf Coast region for a 50 year period? The key obviously has been extraordinary survivorship, a property now severely compromised by chronic Dermo infection and periodic MSX epizootics. Habitat loss has been the result of landward development and atrophy associated with dying oyster populations. The harsh reality is that scaling up current restoration efforts will not restore oyster harvests and/or populations to levels 20, 50, or 100 years ago.

MANAGEMENT-RELATED OYSTER DISEASE RESEARCH IN MARYLAND—AN OVERVIEW. S. J. Jordan,* Maryland Department of Natural Resources, Cooperative Oxford Laboratory, 904 S. Morris St., Oxford, MD 21654.

The Oyster Disease Research Program has been instrumental in helping Maryland develop and implement its comprehensive oyster recovery effort. Work completed or in progress includes: 1) Development of a Microcomputer-based Geographical Information System for the Visualization, Interpretation, and Analysis of Maryland Chesapeake Bay Oyster Disease and Population Information; 2) Technical Support for Maryland's Oyster Recovery Action Plan, and 3) Experimental Management of Maryland's Oyster Recovery Areas. The GIS project has been the foundation for a much expanded database that now includes comprehensive historical and current habitat and management data. The system is used daily for a variety of scientific, management, and public information purposes. The second project has resulted in increased understanding of spatial variations in parasitic oyster infections, along with improved protocols for sampling, diagnostics, and seed oyster certification. The last project has established significant plantings of disease-free hatchery seed oysters in a low salinity sanctuary where they can be monitored for long-term growth, survival, and parasitic infections. Although the objectives of these projects were primarily management-oriented, they have also answered some scientific questions and fostered scientific collabora-

rations. The early decision to support projects addressing both technical and management priorities has contributed to a strong and productive Oyster Disease Research Program.

DEVELOPMENT OF ENHANCED DIAGNOSTICS AND IDENTIFICATION OF OYSTER TARGET MOLECULES FOR *PERKINSUS MARINUS*. S. L. Kaattari,* D. A. Shapiro, T. D. Lewis, and M. Faisal, School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Critical to the analysis of *Perkinsus marinus* infections is the ability to identify its potential virulence factors and host target molecules. Using the newly developed protein-free, chemically defined culture medium, JL-ODRP-3, we have been able to obtain large amounts of purified *P. marinus* extracellular proteins (ECP) in a purified form and consequently used these to produce specific monoclonal antibodies. To date many laboratories have not been successful in the generation of monoclonal antibodies to *P. marinus*. We have identified some unique biological properties of *P. marinus* ECP, and constituents of the culture media, which were largely responsible for detrimental effects of ECP on murine lymphocytes. We then developed methodologies to overcome these difficulties and have increased the efficiency of monoclonal production. A polyclonal diagnostic assay was developed which can be used to quantitatively assess the intensity of *P. marinus* infections. Our results suggest that data obtained by this ELISA-based assay is comparable to the commonly used, Ray's thioglycollate test. In order to identify the host target molecule(s), purified *P. marinus* ECP were conjugated to sepharose beads. The conjugated beads were then incubated with oyster hemolymph samples. It was found that ECP reacted with a number of eastern oyster (*Crassostrea virginica*) hemolymph proteins. Interestingly, when hemolymph samples of the Pacific oyster (*Crassostrea gigas*) were employed, the protein profiles were not affected. Using these newly developed assays and reagents, analysis of hemolymph constituents may be a practical means of assessing clinical cases and elucidating disease resistance mechanisms.

TWENTY YEARS OF SHELLFISH MANAGEMENT ON MARTHA'S VINEYARD—METHODS AND INSIGHTS. R. C. Karney,* Martha's Vineyard Shellfish Groups, Inc., P.O. Box 1552, Oak Bluffs, MA 02557.

For twenty years, the Martha's Vineyard Shellfish Group, Inc., a non-profit consortium of the shellfish departments of six towns on the Island of Martha's Vineyard, has applied innovative aquaculture techniques to the successful management of local public stocks of economically important shellfish species. Annually, ten to fifteen million seed quahogs, bay scallops, and oysters are planted in the Island's bays to augment natural recruitment. The public stock enhancement program includes the operation of the nation's first public, solar-assisted, shellfish hatchery, an onshore shellfish nursery, an onshore remote system for setting oysters, and

deployment of floating field culture nurseries. Genetic shell color tags bred into the cultured stocks provide a means to track survival and measure the success of the stock enhancement efforts. However successful the public aquaculture program has been, it is clear that the public effort's limitations of manpower and funds limit the attainment of maximum productivity from the Island's waters. Maximum production may be realized with private aquaculture. The Shellfish Group recently launched the Martha's Vineyard Private Aquaculture Initiative to train local fishermen in aquaculture techniques and encourage local private aquaculture ventures.

IDENTIFICATION OF POLLUTANT SOURCES CONTRIBUTING TO DEGRADED SANITARY WATER QUALITY IN TASKINAS CREEK RESERVE, VIRGINIA. H. Kantor* and M. W. Rhodes, School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

This study addressed a need to identify sources of fecal pollution impacting the sanitary water quality of the Taskinas Creek Reserve in Virginia. Taskinas Creek is a National Estuarine Research Reserve site located primarily within the York River State Park watershed but surrounded by areas ranging from undeveloped to low density residential. The tidal portion of Taskinas Creek is closed to shellfish harvesting owing to high fecal coliform densities. Following a detailed sanitary survey to locate potential pollution sources, we applied a "suite" of approved and candidate fecal indicators to feeder streams in developed and reserve areas and in tidal Taskinas Creek for one year. The absence of candidate human-specific indicators (sorbitol-positive bifidobacteria, fluorescent whitening agents) and the infrequent occurrence of FRNA coliphages in feeder streams did not implicate human contamination as responsible for elevated fecal coliform counts in Taskinas Creek. Rather, the study revealed a widespread occurrence of the candidate animal fecal indicator, *Streptococcus bovis*, at all locations sampled. The occurrence of *S. bovis* was not correlated with temperature or salinity. Analysis of limited animal fecal samples corroborated dominant feral animals as sources of fecal coliforms and *S. bovis* to marsh waters and feeder streams. *S. bovis* has potential as a direct indicator of animal fecal contamination but its use requires additional validation and improved methods to confirm presumptive counts.

THE POTENTIAL EFFECT OF URBANIZATION ON IRON BIOAVAILABILITY AND THE IMPLICATION FOR PHYTOPLANKTON PRODUCTION. T. Kawaguchi,* Department of Environmental Health Sciences, School of Public Health, University of South Carolina, Columbia, SC 29208; A. J. Lewitus, Baruch Marine Laboratory and Marine Science Program, University of South Carolina, Georgetown, SC 29442.

A favorable habitat for oyster production can be achieved not only by maintenance of the water purity (e.g. reduction of non-point source pollution associated with urbanization), but also by

maintenance of the water integrity (e.g. high food availability for oyster development, growth, and maturation). Iron bioavailability in relation to algal production in salt marsh estuaries has never been discussed due to the abundance of iron in marsh soils. However, iron readily becomes unavailable to phytoplankton under high salinity, oxygenated conditions. Its availability can be enhanced by chelation to dissolved organic matter, and, in this respect, additional inputs of organically-bound iron from forested streams to estuaries may be important in stimulating phytoplankton growth. Here we present the hypothesis that urbanization-associated deforestation can reduce the amount of iron available to estuarine phytoplankton, and that this in turn can adversely affect phytoplankton population. We compared Murrells Inlet impacted by deforestation, a suburbanized estuary, with North Inlet, a forested estuary, with respect to the concentration of dissolved iron, and the effects of iron enrichment on incubated samples of natural phytoplankton communities and cultured species. The potential for iron depletion by phytoplankton was greater in populations transferred to Murrells Inlet water than in those transferred to North Inlet water. Also, the stimulatory effect of iron enrichment on phytoplankton in Murrells Inlet water was taxonomically selective. The potential effect of coastal forest clear-cutting on iron bioavailability, the repercussions for phytoplankton production and composition, and implications to oyster growth are discussed.

SIMPLE LABORATORY TESTS THAT CAN PREDICT THE POTENTIAL PATHOGENICITY OF STRAINS OF *VIBRIO PARAHAEMOLYTICUS* ISOLATED FROM SHELLFISH OF THE WEST COAST OF THE UNITED STATES. C. A. Kaysner,* K. C. Jinneman, C. Abeyta, Jr., and W. E. Hill, Seafood Products Research Center, Food and Drug Administration, P.O. Box 3012, Bothell, WA 98041.

Vibrio parahaemolyticus is a marine bacterium found in virtually every temperate coastal area of the world. This species is isolated more frequently and in greater numbers from water, sediment, and shellfish during the summer months when water temperatures exceed 15°C. The presence of this bacterium does not correlate to indicator organisms traditionally used for shellfish growing area water quality. *V. parahaemolyticus* is the leading cause of bacterial gastroenteritis from consumption of raw molluscan shellfish in the U.S. Some strains produce a thermostable direct (TDH) or a thermostable related (TRH) hemolysin or both, which are factor(s) for causing gastroenteritis. We have identified predictive traits of strains isolated from clinical specimens and from oyster growing areas of the Pacific Northwest. The production of urease and certain somatic serogroups correlate to pathogenicity and the production of TDH; predominantly urease positive, serogroup O:4 (of the 11 groups currently recognized). In the past decade, strains isolated from this area that do not produce urease have not been found to produce either hemolysin nor contain the *tdh* or *trh* gene (determined by colony hybridization experiments). A monitoring system for the presence and the levels of

V. parahaemolyticus in water and shellfish using simple tests may predict the potential of shellfish lots for human illness.

LONG-TERM PATTERNS OF OYSTER SETTLEMENT IN A RELATIVELY UNDISTURBED, HIGH SALINITY SOUTH CAROLINA ESTUARY. P. D. Kenny,* D. M. Allen, and D. Bushek, Baruch Marine Field Laboratory, University of South Carolina, P.O. Box 1630, Georgetown, SC 29442.

Settlement patterns for the eastern oyster, *Crassostrea virginica*, have been studied since 1982 in a high salinity southeastern estuary where oysters form densely populated intertidal reefs. Vertical arrays (three levels) of collecting plates were deployed for consecutive two week intervals from May to November at one site and examined for oyster spat—a previous study demonstrated that factors controlling oyster settlement are operating at the ecosystem or broader spatial level. Within year fluctuations in abundance were large, but early and late season peaks usually occurred within each year. Within and among year differences in settlement and intensity were generally not related to changes in water temperature and salinity, but low recruitment generally coincided with extreme conditions. Variations in other system-wide factors affecting behavior and survival of larvae and newly settled spat are probably more important in controlling intra- and interannual patterns of oyster settlement during average years. Gregarious settlement and competition with other invertebrates for space appear to suggest that biological interactions are important determinants of settlement and early recruitment.

EFFECTS OF TREATMENT, SITE AND TIME OF RESEEDING ON SURVIVAL AND GROWTH IN THE BLUFF OYSTER, *TIOSTREA CHILENSIS* (BIVALVIA:OSTREIDAE) PHILIPPI 1845. J. A. Keogh,* Department of Marine Science, University of Otago, P.O. Box 8, Portobello, New Zealand; G. Bremner and A. W. J. Frazer, Ministry of Fisheries, Private Bag 1926, Dunedin, New Zealand; D. J. Fletcher, Department of Mathematics and Statistics, University of Otago, P.O. Box 56, Dunedin, New Zealand.

The collapse of the Bluff oyster (*Tiostrea chilensis*) fishery in Foveaux Strait, New Zealand in the mid-late 1980s due to the haplosporidian *Bonamia* sp. prompted initiatives to investigate methods for restoring this fishery. Brooding oysters collected from the wild were stripped of larvae which were then settled on uniquely tagged weathered oyster valves. After intermediate culture of either six weeks or six months, spat on these valves were counted and measured, and the valves allocated to one of three treatments (free, caged, or tethered) prior to reseedling at one of twelve sites. Survival and growth were estimated one month after reseedling and bimonthly thereafter for at least 12 months. Eight months after reseedling, free valves showed lower spat survival (5.1%) and growth (5.7 ± 0.8 mm) than either tethered valves (6.2%; 6.6 ± 0.3 mm) or those in cages (9.3%; 6.7 ± 0.4 mm); spat on caged and tethered valves in deeper water showed higher sur-

vival (14.7%) than those in shallower water (7.2%), although their growth was similar. In the month following reseeding massive mortality occurred—this was greater after six months (70%) than after six weeks (45%) of intermediate culture. While the mean number of spat per valve had fallen to between 5–10% eight months after reseeding, two of the deep sites (Dog Island and Bird Island) had over 20% (= ~10 spat per valve) of the reseeded spat surviving with an average shell height of 6.6 ± 0.4 mm. The implications of these results for fishery enhancement are discussed.

EVALUATION OF AN UPWELLING INJECTION FIELD POLISHING SYSTEM FOR ELIMINATION OF FECAL COLIFORMS AND ENTERIC VIRUSES (F+ RNA PHAGE).

M. B. Kilgen* and **E. Melancon**, Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310; **K. Rush** and **R. Malone**, Department of Civil Engineering, Louisiana State University, Baton Rouge, LA 70803.

A natural system using a sand/soil bed in an upwelling injection field polishing system was designed and implemented to remove fecal coliforms, *Escherichia coli* and enteric viruses from wastewater effluents of coastal dwellings. This system treats wastewater by injecting it 15' down into a salt sand/soil marsh adjacent area. This forces it to flow upward through a salt or brackish water sand bed by the hydrostatic pressure difference between the fresh wastewater influent and the salt water. As the fresh wastewater is pushed upward through the sand bed, suspended or particle-associated microorganisms are either adsorbed by the sand surface or die-off, thus reducing their impact to the surrounding estuary. The injection system proved to be extremely effective in removing fecal coliforms, *E. coli*, and RNA F+ coliphage from secondary treatment wastewater influents. Initial loads of fecal coliforms from this injection influent were reduced by 2–4 logs from about 10^{3-4} or higher to 10^1 , 10^0 , or in most cases, to undetectable levels. The system also removed 6 liters of concentrated (10^8 pfu/ml) MS2 RNA F+ phage seeded directly into the injection line as a model for human Norwalk and hepatitis type A enteric viruses. Overall, this upwelling injection system for salt or brackish water coastal dwellings is extremely effective in removing not only the fecal coliform and *E. coli* load from partially treated sewage effluents, but also the enteric viruses which are of greatest concern in actual human health risk.

POLLUTION ABATEMENT THROUGH COMMUNITY INVOLVEMENT. **T. L. King*** Washington Sea Grant Program, N. 11840 Hwy. 101, Shelton, WA 98584.

Pollution abatement to benefit shellfish beaches in rural communities generally centers around nonpoint source pollution. The primary source of nonpoint pollution is generally local citizens. So, why not actively involve them in the solution of the pollution problem? Through innovative education projects in Puget Sound, volunteers are joining the team necessary to battle nonpoint pollution. For example, in Lower Hood Canal, Puget Sound, citizen

volunteers were trained to help with intensive sanitary surveys. It is estimated that the citizens saved themselves over \$11,000 and provided county health staff with over 1,100 hours of volunteer labor by producing the 23,000 charcoal packets needed for the survey. The results of this project not only helped the county health staff achieve their survey goals, but also had many side benefits. Working on the project, citizens became vested in the on-site sewage system survey methodology and outcome of the survey. When neighbors were being tested by county staff, volunteers were able to be an additional source of information about the survey procedures and the need for testing. With the survey nearly complete, volunteers are moving toward prevention of future on-site sewage system failures by promoting and educating their neighbors about on-site sewage system operation and maintenance. With proper education and leadership, many opportunities within the sanitary survey process can be effectively conducted by local citizens. Teamwork is the key to successfully battling human induced nonpoint pollution sources.

MANAGEMENT ALTERNATIVES FOR PRODUCTION OF CRASSOSTREA VIRGINICA IN PERKINSUS MARINUS ENZOOTIC AND EPIZOOTIC AREAS. **G. E. Krantz*** and **S. J. Jordan**, Maryland Department of Natural Resources, Cooperative Oxford Laboratory, 904 S. Morris St., Oxford, MD 21654.

The major constraint to the production of marketable quantities of oysters along the Atlantic Coast south of Long Island Sound in mortality induced by parasitic diseases. Successful propagation of oysters in this zone requires detailed knowledge of the host parasite interactions and continuous survey of oyster and parasitic populations. *Perkinsus marinus* and *Haplosporidium nelsoni* became epizootic in the Maryland portion of the Chesapeake Bay during the last two decades. Mortalities caused by these two parasites have required major changes in oyster management practices. At present, utilization of low salinity areas with low disease infection pressure has produced harvestable shellfish stocks. Management strategies to enhance and supplement recruitment, along with maintaining growing areas free of the infectious parasites, offer the most promise for production of harvestable stocks in the future.

TWO ATTEMPTS AT INTERTIDAL SHELLFISH HABITAT MITIGATION IN NEW ENGLAND. **J. M. Kurland*** NOAA National Marine Fisheries Service, Habitat and Protected Resources Division, One Blackburn Drive, Gloucester, MA 01930.

In northern New England there has been very little experience in the restoration of physically altered shellfish habitat or the creation of new shellfish habitat. Dredging or filling proposals in shellfish habitat are subject to a rigorous regulatory review, but sometimes habitat losses are unavoidable. To prevent a net loss in shellfish habitat, adequate mechanisms must exist to offset further impacts by restoring or creating productive habitat. This presentation evaluates two attempts to mitigate for the loss of intertidal

habitat (primarily for soft shell clams, *Mya arenaria*) through habitat restoration or creation. The first example, located in Searsport, Maine, involved the creation of new clam flats, and illustrates many of the mistakes that can lead to the failure of a mitigation project. The second example, located in Portsmouth, New Hampshire, involved the restoration of impounded intertidal habitat, augmented by the creation of new habitat. Although it is too soon to judge the success of this second example, the approach is far more holistic than the first example, and the initial monitoring results are promising. The presentation concludes with several recommendations for improving mitigation projects and advancing the state of knowledge for successful shellfish habitat restoration and creation. Shellfish mitigation projects can be enhanced by including scientifically sound techniques, quantifiable success criteria, benchmarks to control sites, thorough monitoring requirements, and realistic expectations. Most importantly, to avoid net losses of available habitat, further research is needed to develop reliable techniques for the restoration and creation of shellfish habitat.

THE EFFECT OF HYDRAULIC RAKE ON SOFT SHELL CLAM STOCK ENHANCEMENT. T. Landry,* K. Moran, and H. Kerr, Department of Fisheries and Ocean, P.O. Box 5030, Moncton, NB, Canada.

Research and development work on soft shell clam (*Mya arenaria*) aquaculture in Atlantic Canada is conducted through enhancement activities on private leases. Clams from closed (contaminated) watersheds are harvested and transplanted on barren leases and monitored for growth and survival. The hydraulic rake is used for harvesting the clams and also to cultivate the ground on the planting plots. The planting success of clams on cultivated (hydraulic rake) plots is higher than on non-cultivated plots. The use of the hydraulic rake is also being evaluated as a tool to enhance natural recruitment on a site by modifying the sediment composition and structure. Preliminary results on the recruitment aspect are showing that the impact of the hydraulic rake on the sediment structure is short-term and the recruitment level is slightly higher on cultivated plots. Harvested plots are also being monitored for recruitment in relation to fishing method and seasons.

IMPACT OF SAND DUNE INSTABILITY ON OYSTER HABITAT AND PRODUCTIVITY. T. Landry,* A. Bogen, and D. Booth, Department of Fisheries and Oceans, P.O. Box 5030, Moncton, NB, Canada E1C 9B6.

The reoccurrence of a hole (stemming from the construction of a channel through the dune) in the Maisonette sand dune is being linked to growth, mortality, and recruitment problems in oyster populations from Caraquet Bay. As restoration plans are being considered in the near future, the objectives of this project are to investigate temporal changes in the physical and chemical hydrological characteristics of the Caraquet Bay in relation to various conditions of the sand dune and to investigate possible correlations

between these various scenarios and the productivity of oyster populations. The preliminary results from this research project will provide valuable information on the impact of changes in the dune structure and the micro-habitat it provides to Caraquet Bay, which is at the northern limit of the geographical distribution of the American oyster. These results are also relevant to all the other important oyster producing bays in Atlantic Canada, as they are also protected by sand dunes. Although the role of these sand dunes in the occurrence and productivity of oyster populations is readily recognized by local residents, little scientific information is available on their impact on oyster productivity. This work includes monitoring physical and biological characteristics of the Caraquet. The approach being developed here is to use a numerical hydrological model to simulate the physical and chemical characteristics of the bay under various scenarios and to use the outputs of these simulations to evaluate the changes in oyster productivity using a second numerical model on oyster production (Hofmann et al. 1992).

EXTRACELLULAR PROTEINS OF THE OYSTER PATHOGEN *PERKINSUS MARINUS* AS VIRULENCE FACTORS AND POTENTIAL TARGETS FOR CHEMOTHERAPY. J. F. La Peyre* and M. Faisal, School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The oyster pathogen *Perkinsus marinus* produces many extracellular proteins (ECP) *in vitro* and there is evidence that these ECP are important *in vivo* for the establishment of infection and propagation of the parasite. For example, parasite body burden in oysters administered liposomes containing *P. marinus* ECP and then challenged with *P. marinus*, increased significantly compared to oysters fed liposomes containing only seawater. Moreover, *P. marinus* ECP may also compromise the oyster host defense mechanisms since we have found that proteases purified from ECP inhibit hemocyte activity and can degrade humoral factors *in vitro*. Analysis of these ECP revealed the presence of a battery of hydrolytic enzymes. Using a combination of inhibitors and specific chromogenic substrates, it was possible to identify *P. marinus* serine proteases with chymotrypsin-like and trypsin-like properties. These proteases hydrolyzed a wide range of proteins including extracellular matrix and oyster plasma. The production of these proteases by *P. marinus* may explain the extensive tissue lysis observed in heavily infected oysters. Our data also show that *P. marinus* produces several glycosidases, lipases, and phosphatases which are known to contribute to virulence in other pathogens. Apart from their metabolic roles, this combination of enzymes may be crucial for the virulence and survival strategies of *P. marinus*. The ability of a number of serine protease inhibitors to suppress the propagation of *P. marinus* *in vitro* further suggests that ECP may be potential targets against this deadly pathogen *in vivo*.

EFFECTS OF OYSTER HARVESTING ON THE HABITAT VALUE OF RESTORED OYSTER REEFS: AN EXPERIMENTAL ANALYSIS. H. S. Lenihan,* NOAA National Marine Fisheries Service, Beaufort, NC; C. H. Peterson, University of North Carolina at Chapel Hill, Morehead City, NC 28557.

We conducted a field experiment to test whether various oyster harvesting techniques, dredging, hand-tonging, and diver-collecting, cause differences in (1) oyster reef morphology, (2) incidental mortality to unharvested oysters, and (3) sedimentation rate across experimental restored oyster reefs located in the Neuse River, NC. We also compared catch per unit effort for each harvest treatment in order to evaluate economic efficiency. In order to mimic the effect of an actual harvest season, professional oyster fisherman were employed to conduct harvest treatments and replicate experimental reefs were harvested until no further returns were made. Reefs harvested with dredges experienced the greatest reduction of reef height (-29 ± 12 cm) and highest sedimentation rates compared to other treatments. Incidental mortality was lowest and catch per unit effort was highest on reefs that were harvested by divers. Alteration of the physical structure of oyster reefs caused by harvest disturbance has important ecological implications because reef height controls local hydrodynamic flow, which, in turn, influences recruitment, growth, and survival of oysters. Long-term production of oysters and other ecosystem services performed by oyster reefs are also controlled by interaction between harvest-related reef degradation and reduced estuarine water quality. Our results provide further evidence for why management of oysters and their reef habitat should be transferred from fisheries to ecosystem managers.

JUVENILE OYSTER DISEASE (JOD): PROGRESS REPORT OF EXPERIMENTAL STUDIES. E. J. Lewis,* and C. A. Farley, National Marine Fisheries Service, Cooperative Oxford Laboratory, 904 S. Morris Street, Oxford, MD 21654.

Juvenile oyster disease (JOD) causes heavy mortalities in young cultured oysters, *Crassostrea virginica*, typically less than 30 mm in size. Bacterial and protistan agents have been investigated as causative agents of JOD, but the cause of the disease remains unknown. Successful management of the disease relies strongly on the use of a strain of JOD-resistant oyster, or on early spawning to allow juveniles to attain 30 mm in size before the onset of disease. Repeated attempts of transmitting the disease to susceptible oysters in laboratory studies using infected oysters and material filtered from the water column have been successful. Attempts also have been made to transmit JOD to *Crassostrea gigas* and *Ostrea edulis*, and to *C. virginica* from clams reared in the same facility as JOD-infected oysters. Transmission to other species of oysters showed elevated mortalities, but histological examinations have not been made for comparison to JOD. However, there has been no report of mortalities in *O. edulis* reared near infected *C. virginica*. No evidence of transmission was found from clams to oysters. Seventeen *Vibrio* spp. and 32 other species

of bacteria have been isolated from JOD-infected oysters in our bacterial studies. Commonly isolated bacteria have been recovered nearly as frequently from JOD-infected oysters as uninfected oysters and show no association with the disease. Ciliates have been routinely isolated from JOD-infected oysters and rarely from uninfected oysters. Bacterial and protistan studies are continuing.

A PSP RECEPTOR BINDING ASSAY SUITABLE FOR USE IN SEAFOOD REGULATORY APPLICATIONS AND MONITORING OF TOXIC ALGAE. G. J. Doucette, M. M. Logan,* C. L. Powell,* and F. M. Van Dolah, Marine Biotoxins Program, Charleston Laboratory, National Marine Fisheries Service, Charleston, SC 29412.

More than 20 countries have either established or proposed regulatory limits for paralytic shellfish poisoning (PSP) toxins. However, problems inherent with the AOAC mouse bioassay determination of PSP toxicity, coupled with international pressure to eliminate live animal testing of seafood products, are fueling a search for alternative assay methods. We have developed a receptor binding assay for PSP toxins that is performed in a 96-well microtiter plate and evaluated using microplate scintillation counting technology. This assay exploits the highly specific interaction of PSP toxins with their biological receptor (i.e., voltage-dependent sodium channels), and is based on competing toxins present in a reference standard or sample against [3 H]STX for sodium channels in a rat synaptosome preparation. The receptor binding assay can be completed in ca. 4 hours with samples in hand, and has a detection limit of ca. 5 ng STX/mL in a sample. Quantification of toxicity with the assay corresponds closely to that obtained by mouse bioassay and/or HPLC analysis in a variety of sample matrices, including various shellfish and toxic algae. Our findings indicate that the receptor binding assay has a strong predictive value for toxicity determined by mouse bioassay, and that this approach warrants consideration as a rapid, reliable, and cost-effective alternative to live animal testing for detection and estimation of PSP-related toxicity in seafood and toxic algae. In addition, use of this assay in conjunction with other emerging technologies (e.g., DNA probes), may permit the near real-time monitoring of both the presence and toxicity of harmful algae.

OYSTER REEF RESTORATION: DEVELOPING RELATIONSHIPS BETWEEN STRUCTURE AND FUNCTION. M. W. Luckenbach,* J. A. Nestlerode, and G. M. Coates, Virginia Institute of Marine Science, The College of William and Mary, Wachapreague, VA 23480.

The demise of the oyster resource in Chesapeake Bay has resulted not only in the collapse of a once productive fishery, but also the loss of critical 3-dimensional habitat. Recent efforts to restore oyster reefs have been based, in part, on the value of reef habitat for other species. Impediments to those efforts arise because (1) species assemblages on reefs generally have not been well described, and (2) the relationship between the physical structure of reefs and the development of reef communities has not been

described. We have initiated large-scale, manipulative studies to investigate the relationship between reef structure and the development of resident and transient communities of intertidal reefs near the mouth of Chesapeake Bay. Replicated reef bases, ranging in size from 0.5–2.0 acres, were constructed in 1995 and 1996 using two substrate types: surf clams and stabilized coal combustion by-products. Early development of both sessile and mobile epibenthos and resident and transient finfish assemblages on the reefs are being monitored using benthic samples, pop-nets and diver observations. The ultimate objective of the work is to develop testable hypothesis relating the structure and function of restored oyster reef habitat. We argue that such hypothesis testing is requisite to prudent environmental restoration.

BACTERIOPHAGE BIODEPURATION OF *VIBRIO VULNIFICUS* CONTAINING SHELLFISH. R. B. Luftig* and W. Pelon, Department of Microbiology, Immunology and Parasitology, Louisiana State University Medical Center, 1901 Perdido Street, New Orleans, LA 70112–1393.

Vibrio vulnificus is a marine bacterium commonly found in the warm waters along the U.S. Gulf Coast and also in shellfish. It is acquired in the course of feeding. Although harmless to most humans, *V. vulnificus* can cause serious illness among those with immune problems. Most of the infections are acquired by eating raw oysters. The methods commonly used to rid live shellfish of harmful bacteria do not work with this organism. The prevention of infection has been limited to public announcements and warnings which have resulted in a marked reduction in raw oyster sales, causing a serious economic loss for the shellfish industry. Earlier, we found bacterial viruses that will specifically infect *V. vulnificus*, destroying it. These are harmless to other bacteria, as well as man. We propose that concentrated virus mixtures be added to tanks of oysters, thereby attacking *V. vulnificus*, and reducing their numbers to safe levels. We call this approach "biodepuration," and regard it as a form of biological warfare. Recently, we prepared highly concentrated virus mixtures and performed studies where live oysters were artificially exposed to *V. vulnificus*, after which the virus mixture was added. Both groups were shucked, the flesh pooled, then assayed for the amount of bacteria remaining. We showed significant reductions in the numbers of bacteria in the oysters following treatment with the viruses. Currently, efforts are being made to increase sensitivity for detection. Our goal is to ensure that oysters for raw consumption may be sold as a safe product.

COMPREHENSIVE LAND USE PLANNING AS A TOOL FOR PREVENTING SHELLFISH HABITAT DEGRADATION. S. L. Macfarlane* and P. H. Halkiotis, Town of Orleans Conservation Department, Town of Orleans Planning Department, 19 School Road, Orleans, MA 02653.

Local Comprehensive Plans are being prepared by all municipalities in Barnstable County, MA as part of a regional effort to plan for future growth and development under broad county-side

goals. The resource-rich town of Orleans has committed to produce a resource-based plan that encompasses such diverse aspects as water resources (coastal, surface water and groundwater), wetlands, open space/recreation, housing, economic development, infrastructure and capital improvements, and transportation. Groundwater has been mapped and ten separate watersheds have been delineated leading to three separate estuaries and the Atlantic Ocean. The primary business district which supports both service based businesses and seasonal tourist enterprises is adjacent to one of the most productive shellfish habitats of the town. Three major highways converge in Orleans and lead to the Cape Cod National Seashore, one of the most highly visited national parks in the United States. Single family residences, with 60% year round occupancy, built primarily on one acre lots (3600 sq. meters) have increased since 1970. With 45 miles of shoreline, the town has substantial area classified as water front or water view, the most valuable residential properties in Orleans. Over 50% of the population is 50 years of age or older. Merging the needs of the population, geographic realities, and environmental requirements of the resources into a plan accepted by the residents is daunting, but those perceptive individuals involved in the process recognize that the environment of Orleans is its economy and must be protected.

SHELLFISH HABITAT MITIGATION THROUGH STORMWATER CONTROL: LOCAL EFFORT AND REWARD. S. L. Macfarlane,* Town of Orleans Conservation Department, 19 School Road, Orleans, MA 02653.

Shellfish areas closed to contamination increased sharply in mid-1980's in Orleans, MA, a coastal community in Cape Cod, prompting the town to undertake a drainage remediation program. One primary cause of closures was the number of stormwater pipes and other conduits entering the estuaries from local, state, and private roads. Draining systems were identified, prioritized with respect to shellfish resources, swimming areas and/or anadromous fish runs, and cross referenced with the volume of drainage, the watershed of the system, and the amount of fecal coliform bacteria entering the estuary from the pipe. The two appropriated over \$400,000 for design and construction of five drains with additional funding from a neighborhood association, a corporation (for construction of an innovative system on their property) and the state for retrofitting existing catch basins to provide leaching capability. A water quality monitoring laboratory, staffed by volunteers who obtained samples and performed laboratory analyses, provided pre-construction data and is contributing data for ongoing analyses of the systems. Five additional drainage systems will be retrofitted by the end of 1996 and will include a system called "storm treat."[®] Data analysis has shown a dramatic decrease in bacterial levels at most sites and Meeting-house Pond, closed to shellfishing since 1982, was reopened in December, 1994 as a direct result of this effort.

OYSTER RESOURCE ZONES BASED ON WET AND DRY ESTUARINE CYCLES AND ITS IMPLICATION TO COASTAL RESTORATION EFFORTS IN LOUISIANA, U.S.A. E. Melancon* and T. Soniat, Nicholls State University, Thibodaux, LA 70310; V. Cheramie, Louisiana Department of Natural Resources, Nicholls State University, Thibodaux, LA 70310; J. Barras, NBS, Baton Rouge, LA; R. Dugas, Louisiana Department of Wildlife and Fisheries, New Orleans, LA; M. Lagarde, LUMCOM, Chauvin, LA.

Oystermen and biologists have developed a 1:100,000 scale map (stored in a GIS format) of the oyster resource (habitat) zones within Louisiana's Barataria and Terrebonne estuaries. Four oyster resource zones were established on the premise that when all other conditions are met, the prevailing salinity during "wet" and "dry" periods within a zone will determine subtidal oyster survival. The four water zones are a 48,805 hectare upper-estuary *dry zone*, where oysters are cultivated during dry years, a 66,960 hectare lower-estuary *wet zone*, where oysters are cultivated during wet years, a 104,735 hectare mid-estuary *wet-dry zone*, where oysters may exhibit longterm survival, and a 113,371 hectare lower-estuary *high-salinity zone*, where oysters are bedded for short periods. During the period from 1958 to 1990, water acreage increased 77% in the dry zone, 67% in the wet-dry zone, 41% in the wet zone, and 9% in the high-salinity zone. During this same period, privately leased acreage increased over 180% from 10,063 hectares to 39,526 hectares. Federal and state agencies are addressing Louisiana's wetland loss by diverting relatively large quantities of freshwater from the Mississippi River into the estuaries in an effort to restore historical salinity patterns and to enhance coastal restoration. Diversions have the potential of shifting oyster production away from the outflow. The map is being used by state and federal resource managers as an environmental assessment tool for oyster beds that will be impacted.

RECONSTRUCTION OF A NATURAL OYSTER BAR IN THE CHOPTANK RIVER USING HATCHERY-PRODUCED OYSTER SEED. D. Meritt*, J. Takacs, and G. Baptist, Horn Point Environmental Laboratory, University of Maryland, Cambridge, MD 21613; K. T. Paynter, Department of Zoology, University of Maryland, College Park, MD 20742; R. Pfeiffer, Oyster Recovery Partnership, Annapolis, MD 20676.

The Maryland Oyster Recovery Action Plan calls for the use of disease-free hatchery seed in the reconstruction of oyster bars in specific zones where no oyster harvesting is allowed and no Dermo or MSX-infected seed may be planted. This plan will allow experimental projects to be performed to test how quickly oyster seed will become infected in these areas and how diseases affect oysters in relatively low salinity areas over a period of years. Reconstruction of a natural oyster bar in the Choptank River was initiated in 1995 with the deposition of 100,000 bushels of dredged fossil oyster shell on a 10 acre portion of a natural bar in the Choptank River. This produced a large, hard platform on which the

hatchery-reared seed could be planted. Spat were produced from larvae reared at the HPEL hatchery, held in setting tanks 4 to 10 days after settlement, then moved to nursery sites in the Choptank River. After 4 to 6 weeks at the nursery sites where the spat grew to approximately 15 mm, they were planted on the prepared oyster bar. By October, 1995, the spat had grown to an average height of 28 mm. Heavy freshwater input in February–June 1996 has lowered the salinity at the site to 3 ppt which may threaten the survival of the young oysters. May sampling revealed no additional mortality due to the low salinity. Surveys will continue through 1996 to determine growth, survival, and infection rates of the oysters.

THE FUNCTION OF CREATED INTERTIDAL OYSTER REEFS AS HABITAT FOR FAUNA AND MARSH STABILIZATION, AND THE POTENTIAL USE OF GEOTEXTILE IN OYSTER REEF CONSTRUCTION. D. L. Meyer*, G. W. Thayer, and P. L. Murphey, National Marine Fisheries Service, Southeast Fisheries Science Center, Beaufort Laboratory, 101 Pivers Island Road, Beaufort, NC 28516; J. Gill, U.S. Fish and Wildlife Service, 177 Admiral Cochran Drive, Annapolis, MD 21401; C. Doley, National Marine Fisheries Service Restoration Center, 1315 Eastwest Highway, Silver Spring, MD 20910; L. Crockett, NOAA Chesapeake Bay Office, 410 Severn Ave., Suite 107A, Annapolis, MD 21403.

Two methods of oyster reef creation were tested: cultch additions to lower marsh fringe and use of geotubes "geotextile" as substrate for spat settlement and growth. Sediment stabilization and faunal utilization value of oyster cultch added to the lower intertidal marsh fringe of three created *Spartina alterniflora* marshes (two north—one south-facing) were examined. Significantly higher marsh edge vegetation loss was detected for non-cultched compared to cultched treatments at the south-facing site after a southeast storm. Significantly higher rates of sediment erosion were detected for the non-cultched treatments compared to cultched treatments at the south-facing site after the southeaster, and at the north-facing site after strong boat wake disturbance. Oyster development and reef fauna were measured for cultched and non-cultched treatments and nearby reference reefs. Within eight months we observed significantly higher abundances of the dominant species, *Panopeus herbstii*, *Eurypanopeus depressus*, and *Alpheus heterochaelis* within the cultched treatment compared to reference or non-cultched treatments. *Crassostrea virginica* spat settlement within the cultched treatment was equivalent to that of reference reefs. Tests with geotextile material indicate that it may provide substrate for spat settlement within four months, but at significantly lower abundances than shell substrate. Second year oyster settlement and growth on geotextile is currently being assessed. We conclude that addition of intertidal oyster cultch stabilizes marsh vegetation and sediment within created marshes and provides habitat for macrofauna. Also, the use of geotextile material for oyster reef construction may prove a suitable substrate and provide profile for constructed reefs.

THE USE OF GEOTEXTILE ON OYSTER REEF CONSTRUCTION. D. L. Meyer* and G. W. Thayer, National Marine Fisheries Service, Southeast Fisheries Science Center, Beaufort Laboratory, 101 Pivers Island Road, Beaufort, NC 28516; C. Doley, National Marine Fisheries Service Restoration Center, 1315 East-West Highway, Silver Spring, MD 20910; L. Crockett, NOAA Chesapeake Bay Office, 410 Severn Ave., Suite 107A, Annapolis, MD 21403; J. Gill, U.S. Fish and Wildlife Service, 177 Admiral Cochran Drive, Annapolis, MD 41401.

The substantial decline of oysters in the Chesapeake Bay has had a profound detrimental effect on the economy and ecology of the bay. Because of a substantial and continued loss of oyster habitat due to anthropogenic and natural events, the creation and restoration of oyster reefs are increasingly considered options to offset habitat losses. Most oyster reef creations utilize the placement of cultch or rubble on available bottom. This study was designed to examine the potential use of geotubes for oyster reef construction. Geotubes, filled with clean dredged material and generally used to reduce beach and wetland erosion, may be up to 20 ft in diameter and several hundred feet long. The geotextile fabric retains the dredged material while allowing water to flow through tiny pores. This study examines the efficacy of using geotubes to create aquatic reefs. We postulate that the geotubes would eventually create a viable reef for oysters and other fouling organisms as they settle and affix to the geotube material. Two locations were selected for this study. A subtidal site on the Tred Avon River on Maryland's Eastern Shore and an intertidal site near the Newport River in North Carolina. These areas have annually been observed to have high spat settlement and support substantial oyster reefs. Within four months *Crassostrea virginica* spat settled on geotextile material, but in significantly lower abundances than control treatments. Second year settlement and growth on the geotextile material treatments is being assessed.

CULTURE OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS*, WITHIN A SMALL-BOAT MARINA ON LONG ISLAND SOUND (CONNECTICUT). M. Mroczka,* P. Dinwoodie, and T. Casanova, Cedar Island Marina, P.O. Box 181, Clinton, CT 06460; R. Goldberg, J. Pereira, P. Clark, S. Stiles, and J. Choromanski, NOAA National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460; D. Schweitzer, Marine Sciences and Technology Center, University of Connecticut, Groton, CT 06340; N. Balcom, University of Connecticut, Sea Grant, Marine Advisory Office, University of Connecticut, Groton, CT 06340.

An innovative suspension-culture rack system was designed to evaluate the potential of intermediate aquaculture grow-out of shellfish seed within a marina in Clinton, Connecticut. Conventional dock space in the marina was modified by cutting out sec-

tions of the decking to gain access to the water below. The cut out sections were replaceable allowing normal usage of the dock. Wire-mesh cages (1 m × 0.5 m × 0.5 m) containing four shelves were constructed and suspended below the modified docks. Shellfish seed were contained on the shelves of the cages within flexible plastic mesh bags with temporary closures of slit PVC pipe at the ends. To evaluate the growth potential of hatchery-reared bay scallop seed in the marina environment, approximately 6,000 animals with an initial shell height of 15.5 mm were reared at different densities and in different mesh size cages and bags from June through November of 1995. Survival of scallops in all treatments was very high, averaging about 90 percent. The fastest growing group of scallops reached an average shell height of 54.1 mm by the end of November with many individuals larger than 60 mm. Stocking density and mesh size were inversely proportional ($P < 0.05$) to growth of scallops over a wide range of sizes. Sea-water current flow, temperature and oxygen regimes, and ambient phytoplankton densities were adequate at this location to support substantial growth with low mortality. The potential is excellent for intermediate grow-out of scallop seed at marinas for aquaculture production or seed transplant efforts to restore scallop fisheries to natural habitats.

MARYLAND'S OYSTER RECOVERY PARTNERSHIP: ENVIRONMENTAL AND ECONOMIC RESTORATION. R. M. Pfeiffer,* The Oyster Recovery Partnership, P.O. Box 6775, Annapolis, MD 21401.

After a precipitous decline in oyster harvests, the governor of Maryland convened the Oyster Roundtable. This deliberative body of 40 represented the harvest, environmental, legislative, scientific, and regulatory communities of the State. After deliberations that lasted most of the year, the group reached an agreement that became known as the Maryland Oyster Roundtable Action Plan. A plan that represented a departure from past practice, it called for the creation of a not-for-profit to implement its recommendations. The Oyster Recovery Partnership of Maryland is the non-profit co-venture of watermen, environmentalists, and aquaculturists dedicated to restoring the ecological and economic role of the oyster to the Maryland waters of the Chesapeake Bay. Often viewed as having dichotomous goals, the Partnership conducts its activities in six major tributaries to the Bay that have been designated Oyster Recovery Areas or ORA's. These rivers are then in turn zoned into areas that are either (a) closed to the fishery, (b) open to harvest with limitations on the introduction of oysters, or (c) open to harvest without restrictions. Working with the scientific and regulatory community in its first two years of operation, the Partnership has embarked on several projects that have begun to provide answers to questions about oyster disease, hatchery production, restoration techniques, and the role of the harvest community in the maintenance of a public fishery.

WATER QUALITY MONITORING IN THE INTERNATIONAL ST. CROIX ESTUARY AREA. R. Ranier,* Program Director, St. Croix Estuary Project, St. Andrews, NB, Canada E0G 2X0.

The 185 km (110 mile) St. Croix River and Estuary are international waters shared by Canada (New Brunswick) and the United States (Maine). The waterway is a designated Canadian Heritage River in recognition of its outstanding heritage qualities. Human activities were historically responsible for serious water quality degradation in the river and estuary. Since the early 1970s, however, major investments in pollution prevention and control have contributed to a recovering riverine/estuarine system. One measure of this is the ten-fold decrease in bacterial levels in the lower river between 1976 and 1990. Nonetheless, clam harvesting has been prohibited on the Maine side of the estuary since 1969. On the New Brunswick side, it is permitted at a number of approved, conditionally approved, and restricted flats. In an effort to obtain current water quality data, the St. Croix Estuary Project undertook a three-year monitoring program beginning in 1993. In the first year, monitoring of 37 freshwater, estuarine and marine sites was conducted on a pilot basis. In 1994 and 1995, a full-scale program involving wastewater treatment plant, freshwater and estuarine monitoring was completed. Limited sediment sampling was also undertaken in 1994. In 1995, water overlay monitoring was accomplished at an economically important clam flat. Within the context of a draft comprehensive environmental management plan for the St. Croix Estuary Area, SCEP has identified various steps that would help enhance clam harvesting opportunities. These include reducing bacterial discharges from some wastewater treatment plants on the New Brunswick side of the estuary; developing watershed-based non-point source pollution prevention strategies; exploring the feasibility of a structured on-site systems maintenance program; and constructing pump-out stations for marine vessels.

MOLECULAR MARKERS FOR THE OYSTER PATHOGEN *PERKINSUS MARINUS* AND PRELIMINARY POPULATION GENETIC ANALYSIS. K. S. Reece* and J. E. Graves, Virginia Institute of Marine Science, P.O. Box 1346, Gloucester Point, VA 23062–1346; D. Bushek* and W. Belle, Baruch Institute for Marine Biology and Coastal Research, Marine Field Laboratory, P.O. Box 1630, Georgetown, SC 29442.

The protozoan *Perkinsus marinus* is a pathogen of the eastern oyster, *Crassostrea virginica*, and may present a significant obstacle to the restoration of oyster populations. Recent studies suggest the existence of oyster populations resistant to *P. marinus* and variation in virulence among *P. marinus* strains. Genetic interactions between the host and parasite are poorly understood, but need to be elucidated if restoration efforts are likely to be successful. Information on population genetic structure is available for *C. virginica*, but not *P. marinus*. We developed molecular markers to examine genetic variation within *P. marinus*. Genomic DNA was

isolated from *in vitro P. marinus* cultures and PCR primers were developed to amplify four polymorphic loci. Fourteen *P. marinus* isolates representing the Atlantic and Gulf Coasts of the United States from Long Island Sound, CT to South Bay Laguna Madre, TX were examined. All four loci revealed polymorphisms among *P. marinus* cultures. Analysis of monoclonal cultures derived from individual cells demonstrated that *in vitro P. marinus* are diploid. Furthermore, variation within cultures i.e. among replicate monoclonal cultures) indicated that oysters may be infected by multiple strains. These molecular markers can be employed to elucidate the population genetic structure of *P. marinus*, information necessary to develop effective oyster restoration and disease management strategies.

APPLICATION OF ODRP PROGRAM DEVELOPMENT FUNDS—UPDATE. W. L. Rickards,* Virginia Sea Grant College Program, 170 Rugby Road—Madison House, Charlottesville, VA 22903.

Within each cycle of oyster disease research funding, a small amount has been set aside for a variety of applications related to management of the overall program. This presentation will provide a summary of the uses to which the program development funds have been put.

CONTINUED STUDIES ON THE IDENTITY OF THE JOD CAUSATIVE ORGANISM IN NORTHEASTERN UNITED STATES *CRASSOSTREA VIRGINICA*. E. B. Small,* Department of Zoology, Zoology/Psychology Bldg., University of Maryland, College Park, MD 20704.

In the past two years of study, juvenile oysters were obtained from the Frank M. Flowers Pine Island Oyster Co.'s facility at Oyster Bay, Long Island. Thick sections of infected oyster mantle tissue were carefully prepared for TEM, and examined for intracellular parasites previously shown in mantle epithelial cells by light microscopy (Austin Farley, NMFS, Oxford, MD). These intracellular parasites possessed Fuelgen positive inclusions presumed to be macronuclei and micronuclei. Parasites were not seen in the present thick sections of infected tissue fixed for TEM. The work in 1996 concentrated upon fixing tissues for TEM at the onset of infection in order to obtain infected epithelial cells, which had not been completely destroyed as a result of the infective process. Suspected tissues from the same oyster population have been fixed both from juvenile oysters prior to the time of the maximum level of host mortality, and from juveniles showing classic JOD shell check and conchiolin deposition. Ultimately, thick sections of infected mantle tissue should yield TEM micrographs of the proverbial "needle in the haystack," the suspected protistan parasite *in situ*. Culturing of ciliated protists from the mantle cavity of juvenile oysters with indications of JOD also continues. Under light microscopic examination of live ciliated some of these potential vector ciliates have exhibited morphologi-

cal abnormalities characteristic of infection with an intracellular parasite. Several of these ciliate species are now in culture.

CHESAPEAKE BAY OYSTER REEF—AN EXAMINATION OF RESOURCE LOSS DUE TO SEDIMENTATION. G. F. Smith,* K. N. Greenhawk, and M. L. Homer, Cooperative Oxford Laboratory, and Piney Point Hatchery, Fisheries Service, Maryland Department of Natural Resources, 904 S. Morris St., Oxford, MD 21654.

The chronic decline of oyster harvest in the Chesapeake Bay has been blamed on a combination of three factors; over-harvest, disease, and sedimentation over oyster bottom. Of the three factors, the chronic effects of sedimentation and burial of historic oyster reefs may be the most difficult to quantify on a large scale. An integration of hydro-acoustical and Geographical Information System (GIS) technology was employed to test the assumption that severe sedimentation has been a critical factor in making large expanses of the Maryland Bay non-productive in a harvest sense. Combinations of sub-bottom profiling equipment and side scan sonar were employed over regions of the Maryland Bay previously characterized as oyster bars. Ground truthing of historic bottom was accomplished utilizing results of the 1975–1983 Bay Bottom Survey as well as patent tong survey data from 1989 to the present. GIS grid cell integration of all data types into a standard format allowed for data integration in two- and three-dimensional format. Sediment accumulation over historic oyster bars can clearly be shown. Correspondence of buried basement reef to historic turn of the century charted oyster bars is highly apparent. The effects of sedimentation on oyster shell plantings for rehabilitation purposes can also be clearly demonstrated.

THE GREENWICH BAY INITIATIVE: A CASE STUDY OF SHELLFISH HABITAT RESTORATION THROUGH LAND USE PLANNING. J. Stevens,* M. Pilaro, and D. Geagan, Warwick Planning Department, 3275 Post Road Warwick, RI 02886.

In December 1992, the Rhode Island Department of Environmental Management closed Greenwich Bay's 1,280 acres of highly productive shellfish beds due to dangerously high levels of fecal coliform bacterial pollution. Economically, Greenwich Bay previously accounted for up to 90 percent of the State's winter hardshell clam harvest. Locally, the Warwick shellfishing industry generates \$4–6 million annually and employs over 500 individuals. In response to this environmental and economic disaster, Warwick Mayor Lincoln Chafee directed several City Departments to prepare a strategic plan for the unconditional re-opening of Greenwich Bay to shellfishing. Thus, the "Greenwich Bay Initiative" was established, evolving into a non-partisan association of federal, state, and local government agencies, as well as non-profit environmental organizations and private agencies, combining to work cooperatively to restore Greenwich Bay's health. The ultimate challenge is to revitalize Rhode Island's beleaguered shellfishing industry and restore the health of our marine environment.

The Greenwich Bay Initiative is being carried out through cooperative efforts in four focus areas: coordination, research, remediation, and education outreach. Some of the Initiative's accomplishments include: computer monitoring of pollution levels in the Bay; high-tech GIS mapping and digital orthophotography to identify pollution sources; free sewer connections for low-to-moderate income families; installation of innovative and alternative on-site wastewater technology; installation of best management practices to reduce pollution from stormwater runoff; installation of eight pumpout facilities at Warwick marinas; and watershed training in wise land use practices for municipal board and commission members. The Greenwich Bay Initiative represents public/private partnership at its best. By working cooperatively, the local governments are operating in the most efficient manner possible, primarily by avoiding duplication of effort and through resource sharing. The Greenwich Bay Initiative, through its promotion of unprecedented non-partisan cooperation and resource sharing, is an ideal model of shellfish habitat restoration.

ECONOMICS OF AUGMENTATION OF NATURAL PRODUCTION USING HATCHERY TECHNIQUES. J. E. Supan,* Office of Sea Grant Development; C. A. Wilson, Coastal Fisheries Institute; K. J. Roberts, Louisiana Cooperative Extension Service, Louisiana State University, Baton Rouge, LA 70803.

Investment, fixed, and operating costs of producing oyster seed by remote setting of hatchery-reared larvae were analyzed based on a three-tank setting system operating over a five-month period (May–September). Data were gathered from previous field demonstration projects and interviews with oyster producers. Scenarios were budgeted based on manual labor vs. mechanization, and vessel ownership vs. leasing. Costs per shellbag of seed and potential production of market-size oysters were estimated. The estimates included the purchase of oyster larvae from a hatchery at \$100/million. Mechanized cultch handling with vessel ownership constituted the most cost-effective scenario, with setting and nearshore nursery operating costs comprising 64% of the cost of production, 14% in vessel operation, and 5% in labor (cultch handling). Such a scenario could produce 20 mm oyster seed at approximately 20/shell at a cost of \$6.48/shellbag, averaging 250 shells/bag. A public entity created for seed production and planting on public oyster reefs is hypothesized.

OFF-BOTTOM CULTURE OF OYSTERS USING THE FLOATING CHUB METHOD. J. Swartzenberg* and B. Swartzenberg, J&B AquaFood, 16 East Bayshore Blvd., Jacksonville, NC 28540; S. Kemp,* University of North Carolina Sea Grant, P.O. Box 3146, Atlantic Beach, NC 28512.

In 1995 J&B AquaFood embarked on a two-year pilot study to produce commercial quantities of off-bottom cultured oysters (*Crassostrea virginica*) using efficient agricultural and manufacturing techniques. The project is modeled after the floating chub system developed by Skin Kemp, UNC Sea Grant. Results could

support a cottage industry capable of being accessed by current shellfish leaseholders with capital and a willingness to invest up to three years of effort before realizing a net profit. Seed oysters spawned in a hatchery from supplied brood stock are grown first in flat spat bags and cages which either sit on PVC racks or float using various Styrofoam float configurations. The seed oysters are then transferred in an assembly line basis to tubular mesh bags, called chubs, and floated until harvest. Total time in the water to a marketable oyster ranges from one year to 15 months. A select, clean and meaty, deep cupped oyster is harvested. Controlled comparisons along with numerous taste tests prove the chub grown oyster to be far superior in taste and yield to bottom grown East Coast and Gulf Coast oysters. Initial marketing tests indicate strong potential within the seafood market, grocery, and restaurant trades. Economics are encouraging even at this early stage of marketing and indicate the system is ideally suited to family farm operations where attention to detail and hard work can provide a handsome income. With good management practices, the operation could be easily accommodated within a larger corporate structure.

BAY SCALLOP STOCK RESTORATION EFFORTS IN LONG ISLAND, NEW YORK: APPROACHES AND RECOMMENDATIONS. S. T. Tettelbach,* Natural Science Division, Southampton College, Southampton, NY 11968; C. F. Smith, Cornell Cooperative Extension of Suffolk County, Marine Program, 3059 Sound Ave., Riverhead, NY 11901; P. Wenczel, Long Island Green Seal Committee, 675 W. Shore Drive, Southold, NY 11971.

Populations of bay scallops, *Argopecten irradians irradians*, in waters of Long Island, New York, have been heavily impacted by intermittent blooms of *Aureococcus anophagefferens* ("brown tides") since 1985. The historically valuable fishery has been largely crippled, with concomitant impacts on local fishers and coastal economies. Public restoration efforts by state, county, and local organizations since 1986 have focused on transplanting hatchery-reared and natural scallops to serve as potential brood stock in formerly productive areas. Evaluation of potential sites for transplantation has included dive surveys to examine bathymetric characteristics and predator fields, and analysis of surface current patterns. The approach, which has resulted in the highest survival of transplanted scallops to the time of spawning, is to move individuals from natural stocks in May, a few weeks prior to the anticipated commencement of spawning. Where transplantation of natural stock is not possible, hatchery-reared animals have been reseeded in the fall, when they are available from hatcheries. Overwintering mortality is often severe, but deployment of scallops in lantern nets appears superior to free-planting on the bottom. When free-planting is conducted, we have experience better overwintering survival when scallops are planted at large sizes (>30 mm), at densities <10/m², at sites with greater bottom heterogeneity (e.g.

seagrass, macroalgae, shell hash) which are protected from prevailing winds, and when plantings are done after mid-October. Reseeding efforts using these approaches have been somewhat successful in New York, but permanent recovery of the bay scallop resource has been thwarted by recurring brown tides.

"BAGS TO DRAGS," THE STORY OF THE BAY SCALLOP RESTORATION PROJECT. W. H. Turner,* K. A. Tammi and M. A. Rice, The Water Works Group, P.O. Box 197, Westport Point, MA 02791.

In an effort to focus widespread public attention on the economic value of clean and productive estuaries, The Water Works Group, Inc. founded by Bay Scallop Restoration Project (BSRP) in the Westport River (Massachusetts and Rhode Island). Since its spawning in January 1993, the BSRP has devised and set into action an innovative bay scallop propagation program employing uniquely simple equipment and a large cast of community players. Success in generating viable shellfishing opportunities in Westport has led to the expansion of this initiative to Apponagansett Bay in Dartmouth, Massachusetts. Building on four years of research, investigations have sought to examine the life stages of bay scallops from spawn to settlement in five distinct areas in the Westport River and four in Apponagansett Bay. Experimental results in 1993, 1994, and 1995 have demonstrated the significance of "spawning sanctuaries." These sanctuaries concentrate a broodstock and when coupled with "spat bags" (equipment designed to collect a sample of the juvenile offspring) have recorded volumes of data vital to the understanding of bay scallop recruitment dynamics. Information retrieved from more than 6,000 spat bags have clued researchers into the impacts and significance of: predation (particularly that of mud crabs), fouling organisms, and shellfish spawning events, i.e. larval development, settlement selection, and timing. As a result of this research initiative, three consecutive viable "wild" bay scallop crops have occurred in the Westport River leading to a substantial increase in the number of commercial and recreational scallopers. In turn, this has generated an unprecedented interest focused on improving water quality for further enhancement of commercially valuable shellfish, including oysters, quahogs, and soft-shell clams. Visible and valuable research with an economic end involving public/private partnerships between The Water Works Group and volunteers, local schools, town boards, state agencies, universities, and other nonprofit entities have played a significant role in instilling the vision of this experiment in the minds of thousands of people. So significant is this undertaking, that not only is Westport on the eve of its best scallop season since 1985, but in the four years this project has been drawing attention to the need for high water quality standards, more than 1,200 acres out of 3,000 have been reopened to shellfishing in Westport, allowing resident and commercial fishermen to harvest quahogs, steamers, and oysters deemed off-limits for nearly twenty years.

MOLECULAR BASIS FOR THE ETIOLOGY OF PERKINSUS MARINUS DISEASE AND DEVELOPMENT OF PCR-BASED DIAGNOSTIC ASSAYS. G. R. Vasta,* A. G. Marsh, J. D. Gauthier, A. C. Wright, J. A. Robledo, H. Ahmed, T. J. Burkett, G. M. Ruiz, and C. A. Coss, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E. Pratt St., Baltimore, MD 21202, and Smithsonian Environmental Research Center, P.O. Box 28, Edgewater, MD 21037.

The *in vitro* propagation of *Perkinsus marinus* has allowed us to develop a comprehensive research program on "Dermo" disease that includes the development of molecular diagnostic tools and the study of fundamental aspects of the parasite's biology. We have developed specific and sensitive semiquantitative and competitive PCR assays that will be useful for assessing intensity and prevalence of infections in oyster populations, certifying disease-free spat monitoring of overwintering populations, elucidating mechanisms of infection, and assessing the presence of the parasite in the environment and other invertebrate species. The diagnostic methodology developed is species-specific and the target DNA sequences revealed the presence of at least two sequence patterns with distinct geographic distributions. We have characterized soluble factors (glycoproteins and divalent cations) that promote *P. marinus* proliferation *in vitro* and result in a dramatic increase of intracellular RNA levels, indicative of a sudden change in gene transcription rates characteristic of mitotic activation. The identification of gene products relevant to intracellular survival and proliferation include superoxide dismutases and several glycosidases, currently under study in our laboratory. Against the backdrop of environmental parameters such as temperature, salinity, and iron concentration, *P. marinus* disease is determined by the parasite's adaptations for host specificity, infection, and efficient intra- and extracellular proliferation. Understanding the specific biochemical, molecular, and genetic processes that determine *P. marinus*'s virulence in *Crassostrea virginica* will allow the design appropriate strategies to interdict host/parasite interactions in favor of *C. virginica*.

FLORIDA SHELLFISH CULTURE TRAINING PROGRAMS AND THEIR BENEFITS. D. E. Vaughan,* E. Quisenberry, J. Scarpa, and M. Ednoff, Harbor Branch Oceanographic Institution, Inc., 5600 U.S. 1 North, Ft. Pierce, FL 34946; L. N. Sturmer, University of Florida, Cooperative Extension Service, Project W.A.V.E., P.O. Box 89, Cedar Key, FL 32625.

The way of life for many net fishers of Florida changed radically in July 1995 when a state-wide ban on net fishing went into effect. This, along with reduced natural fisheries, has caused severe local economic depressions. Harbor Branch Oceanographic Institution (HBOI) has initiated a number of shellfish culture training programs for economically disadvantaged workers and displaced fishers. The first program, Apalachicola Bay Oyster Farming Project, trained oyster harvesters in the methods of oyster culture; however, the trainees were not able to obtain shellfish

leases. Project OCEAN (Oyster/Clam Education Aquaculture Network) in the Dixie/Levy county area was successful in securing 4-acre leases for 138 graduates in 1993. Project WAVE (Withlacoochee Aquaculture Vocational Education) graduated 49 participants in June 1996 who are expected to work an additional 98 acres. Ongoing projects on both coasts of Florida are training an additional 100 displaced fishers as clam farmers. Seed clams for these projects are provided by HBOI with funding from the Florida Department of Labor and Employment Security utilizing federal Job Training Partnership Act monies. The economic benefit to the local and state economy is evident; the value of cultured clams in Florida for 1995 was \$5.41 million, a 48% increase from 1993. It is anticipated that 248 million seed clams will be planted by farmers in 1996. One ecological benefit from these programs is that farmers are now stewards and advocates for maintaining the health of the coastal ecosystem due to its direct impact upon their businesses.

BAYNES SOUND STEWARDSHIP ACTION PLAN. D. B. Walker,* Environment Canada, Environmental Protection Branch, 224 West Esplanade, North Vancouver, BC, Canada V7M 3H7.

Baynes Sound is a body of water located on the east coast of Vancouver Island, British Columbia, Canada. The Sound is roughly 30 km in length and lies between the City of Comox to the north and Deep Bay to the south. Baynes Sound is one of British Columbia's prime shellfish culture areas with approximately forty-five percent of the total commercial oyster and clam production of the Province grown in this area. However, in recent years the widespread closure of a number of shellfish harvesting areas in the Sound, due to a variety of point and non-point sources, has threatened the viability of the industry and the livelihood of several hundred people employed in the industry. The problems faced by the growers are serious and challenging but are being met head on through the combined efforts of the community, industry, and government. The Baynes Sound Stewardship Action Plan sets out an approach to addressing the water quality problems in Baynes Sound and promoting community stewardship. Several initiatives are underway that involve the community volunteers in remediation efforts around the Sound including storm drain monitoring, farm fencing and revegetation, and septic care and maintenance education programs.

HISTORY AND MANAGEMENT OF THE U.S. ATLANTIC SURFCLAM FISHERY. J. R. Weinberg,* S. A. Murawski, and F. M. Serchuk, National Marine Fisheries Service, Northeast Fisheries Science Center, Woods Hole, MA 02543.

Atlantic surfclams, *Spisula solidissima*, are distributed along the eastern coast of the United States from the subtidal zone to depths of about 60 m. In 1994, the US surfclam fishery harvested 31,000 metric tons (mt) of meats valued at 42 million dollars ex-vessel, with most of the catch taken from Mid-Atlantic waters

between New Jersey and Virginia. Between 1950 and 1970, annual surfclam landings increased by almost 10-fold (3,500 to 30,500 mt) due to increases in fishing effort, harvesting and processing efficiency, and exploitation of new areas. However, harvests declined by 50% between 1974 and 1976 due to overfishing, followed by a massive mortality of surfclams in summer 1976 caused by wide-spread hypoxic water conditions. Since November 1977, US surfclam fisheries in the EEZ (3–200 mi offshore) have been managed under the Surf Clam and Ocean Quahog Fishery Management Plan of the Mid-Atlantic Fishery Management Council. In 1991, an Individual Transferable Quota (ITQ) system was adopted wherein the annual quota is allocated among participating vessels based on their individual quota shares. Subsequently, harvesting capacity and fishing effort have been rationalized; the number of vessels in the fishery has been reduced by over 50% as quota shares have been bought, sold, and combined on fewer vessels. During the past five years, annual landings have remained fairly stable and the surfclam resource maintained at a medium level of biomass. Long-term monitoring, research, and harvesting strategies have been implemented to achieve sustainable yields.

A DEFENDABLE LONG-TERM STRATEGY FOR OYSTER REEF RESTORATION IN VIRGINIA. J. A. Wesson,* Conservation and Replenishment Division, Virginia Marine Resources Commission, P.O. Box 756, Newport News, VA 23607–0756.

The long and dramatic decline of Virginia's oyster resource is well known and solutions for reversing this trend have been actively pursued. Along with the obvious need to effectively control the harvest to protect the remaining population groups, Virginia is actively testing ways to significantly improve oyster stocks through reef restoration. One very simple, though neglected strategy, has been to very lightly add new cultch material to historic oyster rocks that still exist. Shelling rates of 500 to 1,000 bushels per acre directly on top of live oysters have increased spat settlement from 2 to 10 fold annually. Natural oyster beds have also been restored using a hydraulic excavating machine at one tenth of the cost of conventional shell planting methods. The most intensive restoration attempts have involved the construction of three-dimensional reef structures on historic oyster beds. These structures have been built with shell cultch primarily; however, one new reef now includes a coal ash cultch material. Constructed reefs have begun to provide information which is guiding the formula-

tion of a long-term restoration strategy. Reefs appear to have the most demonstrable effects in small river systems where improved spawning efficiency on the reefs may increase spatset on oyster beds in the proximity of the reef. In one case in the Piankatank River, one reef structure has been associated with increased spatset on natural oyster beds over a 2,500 acre area surrounding the constructed reef. In combination, these three techniques have shown great promise for a long-term strategy for oyster restoration in Virginia.

VESSEL SEWAGE DISCHARGE: ITS IMPACT ON SHELLFISH BEDS AND THE LEGISLATION THAT IT IS MANDATED BY. J. C. Woodley,* USEPA 45004F, Office of Water, 401 M St., SW, Washington, DC 20460.

Commercial and recreational boating plays an important role in American society. Unfortunately, without proper management, these activities can contribute to water quality degradation. One of the specific types of degradation involves the increased concentration of fecal coliform bacteria (found in the intestinal tracts of all warmblooded animals). When concentrations of fecal coliform bacteria rise above safe levels, local health boards act to close swimming areas, as well as restrict or ban commercial and recreational shellfish harvesting. A fecal coliform bacteria count of 14 per 100 milliliters of water results in the closing of shellfish beds. The discharge of untreated or partially treated human wastes from vessels is strongly believed to contribute to high bacteria counts and subsequent increased human health risks. Recently, an outbreak of Norwalk virus gastroenteritis associated with eating contaminated raw oysters was attributed to illegal dumping or discharge of untreated human sewage from shellfish harvesting boats. Vessel sewage discharge is regulated under Clean Water Act (CWA) section 312. Section 312 mandates the use of marine sanitation devices (MSDs), on-board equipment for treating and discharging or storing sewage, on all commercial and recreational vessels that are equipped with installed toilets. Also, under section 312 of the CWA, EPA or States may request a "No Discharge Zone" designation that prohibits the discharge of sewage from all vessels into defined waters. EPA actively supports several efforts designed to increase public awareness about proper use of MSDs, environmental threats from vessel sewage discharges, and the availability of marine pumpout stations.

ABSTRACTS OF TECHNICAL PAPERS

Presented at the 17th Annual Meeting

MILFORD AQUACULTURE SEMINAR

Milford, Connecticut

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OVERVIEW, 17th MILFORD AQUACULTURE SEMINAR.

Walter J. Blogoslawski, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT 06460.

The 17th Milford Aquaculture Seminar provided a unique forum for 157 attendees to meet in both formal and informal settings to exchange the latest developments in aquaculture technology and to make new contacts from business, state, university, and federal venues. Thirty-nine presentations covered topics that included new methods of micro- and macro-algal culture, bay and sea scallop farming projects, problems of exotic species introductions, juvenile and adult shellfish disease, methods to cope with low salinity when growing shellfish, results of some federally funded aquaculture training projects, and an extensive selection of papers devoted to state and federal policy issues relative to leasing of underwater lands for culture purposes and the permitting process necessary to obtain legal use for an aquabusiness. Representatives from forty-eight different aquaculture companies attended the seminar, a record number for this annual event.

The participation of our speakers and exhibitors is greatly appreciated as is the financial support from our sponsors, the U.S. Department of Commerce's National Marine Fisheries Service, Woods Hole, MA and the U.S. Department of Agriculture's Northeastern Regional Aquaculture Center, N. Dartmouth, MA. The cooperation and interest of all who attended made this meeting a lively forum for the timely dissemination of new aquaculture information. Several commercial growers acknowledged that the Milford Aquaculture Seminar was the most important meeting for them to attend each year, the information gained from the meeting being directly beneficial to the "bottom line." In continuing a tradition of speakers and participants drawn from a diversified yet vitally interested group of persons involved in aquaculture ventures, the audience of 157 represented ten states, the District of Columbia, nineteen Universities, forty-eight aquaculture enterprises, nine consultants, and twenty-two marine labs as well as state or federal agencies tasked with aquaculture missions. Their interest and participation made our meeting a great success through the sharing of ideas and new technologies that aid and promote the growth of aquaculture.

A SHELLFISH MARICULTURE TRAINING PROGRAM FOR LONG ISLAND COMMERCIAL FISHERMEN.

John Aldred, Town of East Hampton Shellfish Hatchery, Montauk, NY 11954; **Gregg Rivara**, Cornell Cooperative Extension of Suffolk County Marine Program, 3690 Cedar Beach Road, Southold, NY 11971.

Aquaculture generally has been viewed skeptically by the traditional fisheries in New York. While public enhancement programs have largely been supported as contributing to the overall

welfare of the resource and thus the wild harvester, private aquaculture has been perceived in the context of corporate interlopers whose actions could unravel the indigenous fabric.

Resource and water quality decline, the growth of waterfront development, more stringent shellfish sanitation classifications and increasing regulatory pressures have made wild harvesting significantly less viable in recent years. In some cases, the recent ascendance of public enhancement programs has made aquaculture less exotic and more accessible. An increasing number of fishermen have been coming to view culture as a possible means by which their livelihoods might be augmented and therefore maintained.

Through the East End Institute, funds were made available in 1995 from New York State and in 1996 from the Fishing Industry Grants Program of the National Marine Fisheries Service to establish and then expand a pilot-scale, oyster-culture training program using simple off-bottom culture techniques and encouraging the ingenuity of the participants. The inception, provisions, and progress of the program is discussed.

AN EXPERIMENTAL FEEDING REGIME FOR LARVAL BAY SCALLOPS THAT INDUCES METAMORPHOSIS ON A CONTROLLED SCHEDULE. **Jennifer H. Alix, Mark S. Dixon, Barry C. Smith, and Gary H. Wikfors**, USDOC, NOAA, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, Milford, CT 06460; **Mark S. Dixon**, Marine Sciences and Technology Center, University of Connecticut, Groton, CT 06430.

Previously, we observed early metamorphosis at relatively small size of larval bay scallops, *Argopecten irradians*, when fed the flagellate *Dicrateria* sp., strain CCMP459. To follow up on this observation, we conducted several experiments to explore potential practical applications of this algal strain as a component of larval feeding regimes designed to induce metamorphosis at the convenience of the hatchery operator.

In one experiment, we compared growth and days-until-metamorphosis in larval scallops switched from a unialgal diet of T-ISO to a unialgal diet of CCMP459 on days 4, 5, 6, 7, or 8. The dietary switch on day 6 resulted in metamorphosis on days 8–9 and was considered most promising. In a subsequent experiment, we sought to determine the smallest portion of the T-ISO diet we would need to replace with CCMP459 on day 6 for effective induction of metamorphosis (10, 25, 50, 75, and 100% were tested). The rationale for this experiment was that CCMP459 is relatively difficult to culture. Percentages less than 75% resulted in slower growth and later metamorphosis than diets with 75 or 100%; therefore, a switch to 75% CCMP459 + 25% T-ISO on day 6 was considered to be the optimal larval feeding regime to induce early metamorphosis.

The final experiment was designed to determine whether or not viable post-set scallops were produced using the experimental

feeding regime of T-ISO, changing to 75% CCMP459 + 25% T-ISO on day 6, as compared with a unialgal larval diet of T-ISO. As post-set scallops rapidly lose the ability to filter small cells, such as CCMP459, all scallops were switched to a diet of *Tetraselmis striata*, strain PLAT-P, when metamorphosis was first observed. On day 29 of life (about 20 days after metamorphosis), living post-set scallops (ca. 1 mm) were counted. Approximately 1,500 post-set were recovered from a pre-set population of 400,000 on the experimental feeding regime (only about 0.3%), but this was six times the number recovered (250) from the T-ISO-only regime. Reasons for overall poor setting success probably were related to sub-optimal conditions in the 10-liter buckets employed (temperatures 18–20°C, poor setting surface, and fouling of bucket sides after setting). Nevertheless, we are encouraged by the relative success of the experimental feeding regime and intend to test it in 400-liter conical tanks in collaboration with other Milford programs.

USE OF SPRAY-DRIED SCHIZOCHYTRIUM SP. AS A PARTIAL ALGAL REPLACEMENT FOR JUVENILE BIVALVES. Phil Boeing, Aquafauna Bio-Marine, Inc., P.O. Box 5, Hawthorne, CA 90250.

The use of spray-dried heterotrophically grown microalgae as an aquaculture feed has been previously evaluated. However, these strains were selected more for their heterotrophic culture potential than for their nutritional profile, particularly their $n = 3$ and $n = 6$ HUFA. This paper evaluates the performance of Manila clam spat (*Tapes semidecussata*) and Pacific oyster spat (*Crassostrea gigas*) fed on two different ratios of a spray-dried preparation of heterotrophically grown *Schizochytrium* sp., an algae of very high HUFA concentration. The work consisted of two separate experiments conducted in duplicate. Both experiments tested a 40% and an 80% *Schizochytrium* sp. substitution against a 100% live algae control. In the first experiment, *Tetraselmis suecica* was used as a live algae control of moderate to poor nutritional value. Significantly higher growth over control was obtained with 40% *Schizochytrium* sp. substitution in *C. gigas* and 40% and 80% *Schizochytrium* sp. substitution in *T. semidecussata*. For the second experiment, equal portions of *T. suecica* and *Chaetoceros* sp. were used as live algae control of high nutritional value. The growth rate of both *C. gigas* and *T. semidecussata* controls increased over 600% on the mixed live algae diet as opposed to the first experiment with only *T. suecica*. Significantly lower growth rate was found for *C. gigas* at both 40% and 80% substitution. However, no significant growth difference was found for the *T. semidecussata* at 40% substitution in contrast to a significantly lower growth compared to control at 80% substitution. The results suggested that *Schizochytrium* sp. as a partial replacement for live algae in bivalve culture is economically viable, depending on the unit production cost of the live algae for any given nursery facility.

CULTURE OF MARINE FINFISH AT THE NATIONAL MARINE FISHERIES SERVICE NARRAGANSETT LABORATORY. Lawrence J. Buckley, URI/NOAA CMER Program, Graduate School of Oceanography, Narragansett, RI 02882 and USDOC, NOAA, National Marine Fisheries Service, Northeast Fisheries Science Center, Narragansett, RI 02882.

Research at the NOAA/NMFS/NEFSC Narragansett Laboratory has concentrated on understanding the physical and biotic factors that affect growth and survival of marine fishes during their early life stages. Our work includes: 1) laboratory culture and experimentation; 2) development and application of biochemical and molecular indices of physiological status; and 3) field studies. Over a dozen species have been spawned and cultured at the facility over the past 25 years. These have included Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), silverhake (*Merluccius bilinearis*), winter flounder (*Pleuronectes americanus*), summer flounder (*Paralichthys dentatus*), yellowtail flounder (*Limanda ferrugineus*), Atlantic mackerel (*Scomber scombrus*), scup (*Stenotomus chrysops*), sand lance (*Ammodytes* sp.), tautog (*Tautoga onitis*), striped bass (*Morone saxatilis*), and Atlantic herring (*Clupea harengus*). Recent work has concentrated on winter flounder, cod, and haddock. We currently maintain the only broodstocks of cod and haddock in the United States. Research underway with cod and haddock is designed to extend the portion of the year when first-feeding larvae are available, to describe the seasonal cycle of gamete production in haddock, and to assess the suitability of commercially-formulated diets for juveniles.

REGIONAL LEGAL FRAMEWORK FOR AQUACULTURE. Daniel A. Curran, Marine Policy Center, Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

Per capita seafood consumption in the United States continues to rise, even with the reduction of some wild fish stocks. As a result, most seafood industry analysts fully expect to see production from domestic aquaculture operations responding positively to increased consumer demand in many seafood markets. Although definitions of "aquaculture" are all generally inclusive, normally without regard to the facility location, it is apparent that state and local governments have confined their regulatory emphasis to the coastal regions. While the aquaculture industry is increasing in size in response to the market demand, important legal and policy issues have emerged concerning competing uses of the coastal waters and the regulation of water quality. Human health, propagation and possession permits, import and export regulations, and marketing permits are among the other issues affecting the long term viability of the aquaculture industry, particularly in the Northeastern coastal region of the United States.

The Northeast states have responded to the aquaculture industry in differing fashions. Some, like Connecticut and Maine, have simplified the process to the advantage of the incipient industry, while other states, responding to socioeconomic issues, history,

and politically strong commercial fisheries, have continued a regulatory regime that encourages no more than a "cottage industry" for aquaculture. National restrictions, primarily involving navigation and clean water, further exacerbate the problem. Failure of the national government to update the National Aquaculture Act last year has generally surrendered the aquaculture regulatory responsibility to the individual states. On the other hand, the United Nations Food and Agriculture Organization recently issued a Code of Conduct for Fisheries that includes a section on aquaculture, a positive step toward responsible aquaculture operations in the international market. Many problems remain. It is incumbent on the states, particularly in the Northeast, to attempt to harmonize the various regulations to simplify the regulatory process for the aquaculture industry.

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INTERNATIONAL COLLABORATIVE STUDY OF EEL CULTURE. John J. Curtis, Elizabeth A. Kranyik, and Paul J. Tripp, Bridgeport Regional Vocational Aquaculture School, 60 St. Stephens Road, Bridgeport, CT 06605.

In an effort to foster a marine educational relationship with Shanghai Fisheries University, People's Republic of China, and as a result of a recently signed agreement of cooperation between the two facilities, the Bridgeport Regional Vocational Aquaculture School seeks to expand its curriculum with a proposed two-year study of the eel (*Anguilla rostrata*).

With a recently approved grant proposal, funding for the project has been established to support the study over two phases and through to the end of June, 1988. Phase One will focus on communications with Shanghai Fisheries University to solicit and select two interested/qualified specialists actively involved in their own eel culture research. One of the selected eel research scientists must also have the skills necessary to translate the University's eel culture curriculum from the Mandarin dialect to English for the purpose of its infusion into the ever-expanding curriculum of the Bridgeport Regional Vocational Aquaculture School. It is planned for this scientist/translator to be the first to travel to the United States and work at the school approximately four months prior to the arrival of the second eel specialist. It will be the scientist/translator's responsibility to translate the documents and to assist in the preparation of the Aquaculture School's laboratory for the arrival of the second research person and Phase Two activities. The goals of Phase Two will be to fully involve the students in the study, set-up, capture/grow-out, and marketing of eels.

This project follows the completion of the Bridgeport Regional Vocational Aquaculture School's successful two-year study of suspension culture of bay scallops in Long Island Sound. The project, using funds from Connecticut's Long Island Sound License Plate Program, produced two successful harvests of bay scallops and was supported by the presence of Dr. Luning Sun from Academia

Sinica, People's Republic of China. Students of the Aquaculture School were involved in activities from the set-up of a complete scallop hatchery to the harvest and marketing of the product. The marketing component of the project is being assisted by the Tallmadge Brothers Oyster Co. of Norwalk, CT.

The upcoming study of eel culture looks to continue the professional/educational relationship between the Bridgeport Regional Vocational Aquaculture School and Shanghai Fisheries University for the benefit of aquaculture and those students who choose to pursue aquaculture as a career.

TRAINING AQUACULTURISTS FOR THE 21ST CENTURY: HARBOR BRANCH'S AQUACULTURE EDUCATION AND TRAINING PROGRAMS. Megan Davis-Hodgkins, John Scarpa, and David E. Vaughan, Harbor Branch Oceanographic Institution, Inc., 5600 US 1 North, Ft. Pierce, FL 34946.

The Aquaculture Division of Harbor Branch Oceanographic Institution (HBOI) has been the focal point for hands-on aquaculture training programs in Florida for the past decade. These programs were initiated in response to local economic downturns due to decreases in natural fisheries, government mandated closures of fishing grounds, and a recent ban on net fishing in Florida.

The primary retraining program, funded by the Florida Department of Labor and Employment Security, in Dixie and Levy Counties has retrained over 200 displaced fishers to be clam farmers. These farmers currently work over 700 acres of state-owned submerged lands. Statewide, the value of cultured clams in 1995 was \$5.41 million. Additional projects on both coasts of Florida are training an additional 100 displaced fishers as clam farmers. It was estimated by the state that Florida clam farmers planted approximately 250 million seed in 1996. HBOI-Aquaculture supports this industry by producing clam seed for the growers in the largest US clam hatchery.

Presently, the increased demand for seafood, static wild fisheries landings, and the industry's need for trained aquaculturists has prompted HBOI-Aquaculture to create the Aquaculture Center for Training, Education and Demonstration (ACTED), a hands-on learning facility. This Center provides applied training in marine and freshwater molluscan, crustacean, and finfish aquaculture. ACTED occupies the central six acres of HBOI's 40-acre Aquaculture Development Park. The Park provides a centralized area where industry, researchers, government, and educators collaborate on improving existing aquaculture technology, transferring new technology, and developing culture techniques. The goal of HBOI's education and training programs is to revitalize fishery dependent communities, teach participants an environmentally friendly and sustainable industry, and transfer the latest aquaculture technology.

3H, PH, AND AUXOSPORES—CAN WE MAKE THIS BUG A RELIABLE AQUACULTURE FEED? Mark S. Dixon and Gary H. Wikfors, USDOC, NOAA, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, Milford, CT 06460; Mark S. Dixon, Marine Sciences and Technology Center, University of Connecticut, Groton, CT 06430.

A clone of the diatom, *Thalassiosira pseudonana*, known as "3H", has been used widely in marine research and as an aquaculture feed for larval bivalve mollusks. The positive attributes of this strain include rapid division rates (ca. 1.5 per day), small size, and good nutritional biochemical composition. There is, however, one aspect of this diatom that makes it somewhat unreliable in culture—a reproductive stage in the life cycle that sometimes interferes with regular production schedules.

As do all diatoms, 3H produces resting stages, called auxospores, as a consequence of the cell size reduction that accompanies cell division. In nature, these resting spores serve as seeds for regenerating populations following a period of poor environmental conditions. In this respect, delayed germination of auxospores is a survival strategy; however, when hatchery feeding schedules depend upon expected rates of vegetative growth, spore formation becomes a disadvantage in the hatchery.

We maintained two identical carboy cultures of 3H with weekly semi-continuous harvest and replacement of 1/3 of the 18-liter volume for one year. We observed cycles in relative counts of auxospores and vegetative cells that appeared to be related to pH of the cultures. The pH decreases in cultures with more auxospores are consistent with decreased carbon uptake at a constant carbon-dioxide supply rate. We tested a strategy of decreasing carbon-dioxide supply rate when auxospore counts increased; this resulted in pH increases, which seemed to encourage auxospore germination. This finding suggests that an automated feedback loop in which decreased optical density (fewer vegetative cells), or a decrease in pH, actuates a decrease in carbon-dioxide supply rate and would improve the stability and dependability of continuous or semi-continuous cultures of 3H.

LIVE SHIPPING OF AQUATIC PRODUCTS IN THE NORTHEAST. John W. Ewart, Delaware Aquaculture Resource Center, Sea Grant Advisory Service, College of Marine Studies, University of Delaware, Lewes, DE 19958.

States in the northeastern U.S. have a long history of shipping live aquatic products to regional, national, and international markets. This introductory review provides estimates of market volume and value for leading species traded live in the seafood, recreational sportfishery, and aquarium/ornamental markets. Other topics discussed include regional development of Asian restaurant, retail and international export markets, factors affecting northeastern live markets, and fishery resource issues related to recent trends in live shipping activity.

JUVENILE OYSTER DISEASE RESISTANCE STUDIES CONTINUED: 1994–1996. C. Austin Farley and Earl J. Lewis, USDOC, NOAA, National Marine Fisheries Service, Southeast Fisheries Science Center, Cooperative Oxford Laboratory, Oxford, MD 21654–9724; David Relyea and Joseph Zahtila, Frank M. Flower Co., Oyster Bay, NY 11771; Greg Rivara, Cornell University, Cooperative Extension, Southold, NY 11971.

This is the third year of juvenile oyster disease resistance studies in *Crassostrea virginica*. The first year showed up to 7 times better survival of progeny of a brood stock selected on the basis of (1) survival and (2) presence of characteristic shell checks. The second year evaluated F₁ and F₂ progeny against progeny from susceptible brood stocks deployed in 7 different sites. Survival of F₁ and F₂ resistant seed was 7 to 25 times better than the susceptible seed.

In 1996, we developed an F₃ generation and compared it with the F₂ progeny (FMF) from Flower Co. brood-stocks and a comparable aged susceptible control population from naive natural Connecticut brood-stocks (FCT). Surviving 1995 FCT progeny were used as brood-stocks to produce an F₁ resistant FCT strain. Seed were deployed in 5 different sites in Long Island waters: site 1—Oyster Bay, Long Island Sound, FCT-F₁ (were not deployed here); site 2—Mattatuck Inlet, Long Island Sound; site 3—Cedar Beach, Peconic Bay; site 4—a tidal pond, Peconic Bay; and site 5—Moriches Bay, Great South Bay.

Mortality results after 11 weeks of exposure were in: site 1—(<20 mm) F₂ 20%, F₃ 16%, and FCT 79%, (unculled) F₂ 6%, F₃ 0, and FCT 55%; site 2 (unculled) F₂ 3%, F₃ 9%, FCT F₁ 2%, and FCT 70%; site 3—(unculled) F₂ 15%, F₃ 26%, FCT F₁ 12%, and FCT 65%; site 4 (unculled) F₂ 55%, F₃ 29%, FCT F₁ 23%, and FCT 84%; site 5 (unculled, 9 weeks exposure) F₂ 12%, F₃ 0, FCT F₁ 5%, and FCT 0. Survival was 2.5 to 35 times better in the resistant populations. No significant differences were seen between any of the resistant populations.

Management strategy using resistant seed has resulted in increased production to above pre-JOD levels and has eliminated the devastating effects of this disease.

COMPARISON OF FIELD NURSERY METHODS FOR THE NORTHERN QUAHOG, *MERCENARIA MERCENARIA*, IN COASTAL NEW JERSEY ESTUARIES. George E. Flimlin, Jr., NJ Sea Grant Marine Advisory Service, Toms River, NJ 08755; John N. Kraeuter, Rutgers Haskin Shellfish Research Laboratory, Port Norris, NJ 08349; Stephen Fegley, Maine Maritime Academy, Castine, ME 04421; Steven Mastro, Mastro Clam Farms, Absecon, NJ 08201; George W. Mathis, Jr., Mathis and Mathis Enterprises, Tuckerton, NJ 08087; Peter McCarthy, Peter McCarthy Wholesale Clams, Manahawkin, NJ 08050.

Seed clams (*Mercenaria mercenaria*) are a major cost for shellfish planters. Buying small hatchery seed and conducting the nursery in the estuary can reduce seed costs. Experiments were designed to examine the biological and economic feasibility of grow-

ing seed clams to planting size (8–12 mm in length) in polyethylene (poly) and nylon mesh bags in commercial conditions, and performed over three years. Stocking densities ranging from 2,000 to 6,000 5 mm seed per bag (0.5 to 1.4 clam per square centimeter) were examined first. No density effects were found. Then two types of mesh bags, poly and tented nylon, were examined. Poly bags were deployed both on- and off-bottom, nylon bags were placed on-bottom. Each combination was deployed at two sites in six replicates. Poly bags were filled with 3,000 seed per bag, and the nylon received 6,000, since they were effectively twice the area of the poly. Seed were grown for approximately 2.5 to 3 months and total volume of clams recovered between the sites. Within a site, no differences in seed size could be ascribed to bag type or location. At both sites, the poly bag produced superior survival. Poly bags had similar volumes of clams on- and off-bottom at one site. At the site with lower growth rates, off-bottom clam volume was significantly greater. Economic analysis indicates that on-bottom plastic mesh bags are a viable field based alternative for a hard clam seed nursery.

SEA SCALLOP AQUACULTURE IN MASSACHUSETTS—STATUS AND POTENTIAL. **Robert D. Garrison, Frank Dutra,** and **Scott Feindel**, Harborlife, Inc., 0 Easton Street, Nantucket, MA 01554; **Judy Dutra**, Truro Aquaculture Project, 43 Shore Road, N. Truro, MA 02652; **Richard Taylor**, Gloucester Aquaculture Project, 33 Commercial Street, Gloucester, MA 01930.

The current status of sea scallop aquaculture in Massachusetts includes hatchery activities, spat collection, commercial growout development, and regulatory initiatives. Advances have been made in hatchery techniques, adaptation of culture systems originally developed for the bay scallop, and gear design. The potential for sea scallop aquaculture includes enhancement, offshore aquaculture sites, and the use of former fishing vessels.

Seed produced at the Nantucket Marine Laboratory were successfully deployed at the Truro Aquaculture Project site at less than 2 mm in size using techniques previously developed at the Nantucket Marine Lab for bay scallops. Survival was high with growth rates similar to seed deployed at a larger size. Bottom-cage design was improved by cooperative effort between the authors. Current indications are that cages offer a higher cost benefit and reduced conflicts with other users than suspended culture techniques.

Spat collection and growout experiments were undertaken at Gloucester through a NOAA-sponsored project. The current status of this project will be reviewed.

Fishing restrictions placed upon the sea scallop and current demonstrated advances in aquaculture demand further effort to develop sea scallop aquaculture for enhancement and commercial purposes. Efforts are underway to resolve regulatory constraints.

ADDRESSING PUBLIC POLICY ISSUES ON SCALLOP AQUACULTURE IN MASSACHUSETTS. **Harlyn O. Halvorson**, Policy Center for Marine Biosciences and Technology, University of Massachusetts Dartmouth, North Dartmouth, MA 02747.

A Sea Scallop Working Group (SSWG) representing some 80 individuals from diverse organizations have been meeting since December 1994 to explore critical issues, define possible options for action, develop an industry-driven, bottom-up approach to sea scallop aquaculture, and take advantage of the indigenous and abundant giant scallop, *Placopecten magellanicus*, in Massachusetts waters. A workshop was held (July 24–25, 1995) which involved many individuals representing all aspects and disciplines that would be associated with the development of a sea scallop aquaculture industry in Massachusetts, and used external experts to develop an overall consensus action plan. The principal issues addressed were: sea scallop culture technologies appropriate for Massachusetts; citing criteria, including consideration of user conflicts; potential environmental impacts of sea scallop aquaculture; regulatory restraints to sea scallop aquaculture; economic feasibility of sea scallop aquaculture; public education with respect to sea scallop aquaculture; and developing a better knowledge base for sea scallop biology and aquaculture technology.

The document resulting from this Workshop puts forward recommendations from the perspective of potential sea scallop farmers tempered by the advice and guidance of professional scientists, government managers, regulators, lawyers, environmentalists, and economic development specialists. The major topics which have been subsequently addressed by SSWG will be discussed. These include demonstration projects in federal waters, responses to the Massachusetts Strategic Plan for Aquaculture, recommendations for the New England Fisheries Management Council Development of an Aquaculture Policy and Management Strategy, coalition building, public information and education, GIS technology to identify suitable sea scallop aquaculture tracks in Massachusetts waters, and the Right Whale and other legal considerations.

THE NORTHEASTERN REGIONAL AQUACULTURE CENTER: AN UPDATE. **Kim E. Harrison**, Northeastern Regional Aquaculture Center, University of Massachusetts Dartmouth, 285 Old Westport Road, North Dartmouth, MA 01947–2300.

The Northeastern Regional Aquaculture Center (NRAC), headquartered at the University of Massachusetts Dartmouth, is one of five Regional Aquaculture Centers (RACs) established by the U.S. Congress. Funded by the U.S. Department of Agriculture at an annual level of approximately \$750,000, and representing 12 states and the District of Columbia, NRAC develops and sponsors cooperative regional research and extension projects in support of the aquaculture industry in the northeastern United States.

A Board of Directors representing the region's aquaculture industries, academic institutions, and government agencies provides overall direction and management of NRAC. NRAC programs, like those of all the RACs, are industry-driven; i.e., industry annually communicates research and technology transfer priorities to NRAC through the Center's Industry Committee of the Technical-Industry Advisory Council (TIAC). In assessing priorities, NRAC works closely with State Aquaculture Associations. Projects supported by NRAC are developed and carried out by Cooperative Regional Work Groups (such as the Regional Extension Project) representing a team of highly qualified researchers, extension specialists, and industry representatives who agree to work together to address the industry priorities and by an annual RFP process. All projects include funding for technology transfer. NRAC's Technical Committee provides technical oversight. Projects are evaluated annually for achievement of technical and industry objectives.

NRAC is presently supporting twenty regional projects. Project areas include the development of an extension network and a model quality assurance program, oyster disease, Atlantic halibut, summer flounder, hard clam winter mortality, predation, enhanced digestibility and food conversion efficiency of fish feeds, production of salmon sausage, training for aquatic animal health services, cost effective anti-biofouling surfaces, and the production of an aquaculture awareness video. Total NRAC funding commitment to projects in progress or pending exceeds \$3 million. NRAC also publishes "Northeastern Aquaculture," a quarterly newsletter highlighting NRAC projects and other topics of interest to the northeastern aquaculture community, and has a Home Page on the World Wide Web: <http://www.umassd.edu/specialprograms/nrac>.

GOVERNANCE OF ACCESS TO PUBLIC NATURAL RESOURCES: A COMPARISON OF FEDERAL SYSTEMS.

Porter Hoagland and Di Jin. Marine Policy Center, Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

We present a comparison of federal systems to access to natural resources. The term "access" refers to the legal right to explore, develop, or produce natural resources in the public domain through the sale of resources or land or through lease, license, or other permissions. We can characterize an access system at two distinct levels. At the management level, decisions are made about the size of a royalty or rental, the duration of access, and whether or not an access right can be transferred, among other things. At the governance level, decisions are made about the balance of uses and interests in an area that is potentially subject to access for a specific kind of resource development. We compare access systems at the governance level, identifying both positive and negative aspects. We relate past experience to the case of access to the U.S. exclusive economic zone (EEZ) for offshore aquaculture operations.

FINANCIAL CAPITAL FOR AQUACULTURE: PROBLEMS AND PROSPECTS. **Denise Jarvinen,** Marine Policy Center, Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

According to the Food and Agricultural Organization of the United Nations, global aquaculture production grew at an annualized rate of 9.4 percent per year from 1984 to 1994. As global aquaculture production continues to grow, rates of growth differ across political boundaries. Among other factors, financial capital constraints are cited as limits to aquaculture in certain emerging or expanding sectors. This paper examines the role of government policy in encouraging capital availability for aquaculture expansion and development. Different sources and structures of government support for aquaculture in selected developed nations, U.S. states, and Canadian provinces are described and compared.

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BEATING TRAWLS INTO CAGES—A PROGRAM TO HELP DISPLACED FISHERMEN MAKE THE OCCUPATIONAL TRANSITION INTO AQUACULTURE. **Richard C. Karney, Elizabeth F. Scotten, Gabriella C. Castro, and Debra L. Colombo,** Martha's Vineyard Shellfish Group, Inc., Box 1552, Oak Bluffs, MA 02557.

In 1995, under funding from the National Marine Fisheries Service (NMFS) Fishing Industry Grants (FIG) Program and a NMFS grant to the Nantucket Research and Education Foundation, the Martha's Vineyard Shellfish Group (MVSG) launched the Martha's Vineyard Private Aquaculture Initiative.

The Martha's Vineyard Private Aquaculture Initiative is a comprehensive program designed to help displaced fishermen make the occupational transition into aquaculture. The Aquaculture Initiative hopes to address the chronic high seasonal unemployment on Martha's Vineyard exacerbated by the decline of wild fish stocks on George's Bank and the subsequent fishing area closures by developing local, private aquaculture ventures. To this end, a shellfish aquaculture training program was developed, fishermen were trained, aquaculture development assistance has been secured, socio-political impediments to private aquaculture have been addressed, and in the process, public stocks of shellfish have been enhanced.

Sixteen fishermen negatively impacted by the fishing closures on George's Bank have been given extensive training in practical shellfish aquaculture techniques with the goal of providing alternative employment opportunities in aquaculture. An ambitious twelve-month training program, tailored to meet the needs, interests, and schedules of the displaced fishermen, was designed and implemented. Through the innovative training program, which incorporated lectures, field trips, aquaculture literature, hands-on training in a shellfish hatchery, onshore shellfish nursery, and field

culture sites, fishermen were trained and encouraged to pursue new careers in shellfish aquaculture. The fishermen were trained within the framework of local public stock enhancement programs. The labor they provided increased the effectiveness of these programs, ultimately improving standing stocks of shellfish for harvest. The public onshore shellfish nursery outfitted in this project doubled the onshore culture capacity of the MVSG and will improve public seeding efforts for many years to come.

Aquaculture appears to be an ideal occupational alternative for displaced fishermen finding ready application of their existing water-based skills and producing the same seafood products that they are experienced in handling and marketing. The enthusiasm of the fishermen trainees for this new technology has been overwhelming! Fishermen trainees, polled at the completion of the program, rated the program as "extremely valuable". They overwhelmingly agreed that aquaculture would be "my major source of employment", or would "supplement my employment" in the next five years. The real test to the success of their training will be their ability to put that training into practice and develop economically sustainable aquaculture enterprises. The startup assistance provided under the second round of the FIG program (January 1996–June 1997) will provide the opportunity for these fishermen to put their training into practice.

Private aquaculture ventures can maximize shellfish production, increase the exploitable stocks, and provide alternative employment opportunities for fishermen now competing for limited public shellfish stocks. Retrained fishermen associated with this project are in the vanguard of a movement that could revolutionize the fishing industry, hasten the restoration of natural stocks, and provide unlimited growth potential to the depressed seafood industry.

THE CONNECTICUT STATE DEPARTMENT OF AGRICULTURE—BUREAU OF AQUACULTURE LABORATORY: WHO WE ARE AND WHAT WE DO. John J. Karolus, Stacey L. Spear, and John Volk, Connecticut Department of Agriculture, Bureau of Aquaculture, 190 Rogers Ave., P.O. Box 97, Milford, CT 06460.

Opening in March, 1995, this FDA-certified laboratory is the main facility in the State of CT responsible for the testing of sea water and shellfish for the Interstate Shellfish Sanitation Committee/National Shellfish Sanitation Program. In addition to the routine testing for the presence of fecal coliforms and Paralytic Shellfish Poison (PSP), we decided to look at the prevalence of the protozoan *Perkinsus marinus* in the oyster population along the Connecticut coast. Random samples were taken from May, 1996 through December, 1996 from various locations. In addition, a more controlled look at oysters from seed areas in Greenwich also occurred during this period. Our results revealed that this protozoan pathogen is alive and firmly established in every seed area and cultured bed tested. This confirms earlier published works. The prevalence and weighted prevalence followed typical seasonal

patterns of increasing during the warmer, summer months and slowly decreasing during the early winter. Historical data indicates that this pathogen is a recent addition to the area and its prevalence and weighted prevalence raises interesting questions for the future.

SEA SCALLOP ENHANCEMENT AND CULTURE IN NEW ENGLAND. Sue Kuenstner, New England Fisheries Development Association, 451 D Street, Boston, MA 02210; Richard Langan, University of New Hampshire, Jackson Estuarine Laboratory, 85 Adams Point Road, Durham, NH 03824; G. Jay Parsons, Marine Institute of Memorial University, St. John's, NF, A1C 5R3, Canada; Sandra E. Shumway, Natural Science Division, Southampton College, Long Island University, Southampton, NY 11968; Mark Simonitsch, Fish Weirs, Inc., 84 Doane Road, Chatham, MA 02633.

In early 1996, a sea scallop (*Placopecten magellanicus*) aquaculture project was begun to investigate spat collection and growth of juveniles in various areas of New England. The study sites have been set up and analysis of preliminary results will begin in the spring of 1997. Growth rates of juvenile scallops held in pearl nets and benthic cages will be determined at three sites. In the event that toxin-free scallops are produced, whole and/or roe-on scallops will be test marketed. Spat collection efforts are ongoing at both coastal and offshore sites. Enhancement of local scallop populations through spat collecting activities will be investigated. Spat removed from collector bags after three months and held in upwellers will be compared to those which overwinter on the collectors, to determine if hardier seed scallops can be produced by maintaining spat in a "nursery." An on-board spat sorting system will be developed as a means of decreasing handling and mortality of animals.

REMOTE SETTING OF THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) ON NATURAL AND ARTIFICIAL CULTCH. Richard Langan, Jackson Estuarine Laboratory, University of New Hampshire, Durham, NH 03824; David Gress, Department of Civil Engineering, University of New Hampshire, Durham, NH 03824; Ian Walker, Aquaculture Resource Development, Madbury, 03824 NH; Peter Flanigan, Green Flash Fisheries, Rye NH 03870; Jay Sheehy, F/V Catherine J, Newfields, NH 03856; Jonathan Drake, Jonathan Drake, Rye, NH, 03870; Ken LaValley, Spinney Creek Shellfish, Eliot, ME 03903.

With support from the National Marine Fisheries Service Fishing Industry Grants Program, remote setting trials were conducted using hatchery-produced larvae of the Eastern oyster (*Crassostrea virginica*) on natural and artificial cultch. The setting trials were a component of a comprehensive program designed to provide commercial fishermen with a part-time alternative to wild harvest fisheries.

The natural cultch consisted of bagged oyster shell, sea scallop shell, and whole and broken surf clam shell, while the artificial cultch consisted of the French spat collectors known as "Chinese

Hats'' and an experimental cement-based material designed to dissolve in 12 to 15 months post-set. Spat on natural shell cultch was bottom planted immediately post-set, while the Chinese Hat collectors were suspended from a float for three months prior to removal and planting. Settlement success, growth and survival of the spat are discussed, as well as an economic assessment of the methods used.

AN UPDATE ON THE STATUS OF QPX INFECTIONS OF QUAHOGS IN MASSACHUSETTS. Dale F. Leavitt, Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, MA 02543; Roxanna Smolowitz, LAAMP, University of Pennsylvania, MBL, Woods Hole, MA 02543.

QPX, a Labyrinthomorphid parasite, was first diagnosed in Provincetown and Duxbury (MA) harbors in the fall of 1995. More recently, in 1996, QPX was found during a routine disease examination of hard clams from Virginia. Upon infection, QPX forms thalli surrounded by an ectoplasmic net. The thalli reproduce by forming sporangia through endosporulation. Quahogs respond to the presence of QPX with intense granulomatous inflammation. The QPX infection in the clam was localized predominately in the mantle edges, gill, and connective tissue/sinusoids of the soft body, 91%, 63%, and 27%, respectively. Field evaluations of quahogs infected with QPX indicated a mean mortality of 30% and those animals demonstrating clinical signs of the disease had a significantly reduced condition index.

Examination of seed from four commercial hatcheries routinely supplying seed to Massachusetts growers has indicated that QPX is not routinely transferred via seed transport. In two unrelated mortality incidences on Cape Cod involving young-of-the-year and 1-year-old seed, QPX was not present in the clam tissues. A temporal evaluation of the pathogenesis of QPX infections indicates that detection of the parasite in newly planted seed clam population occurs one year after exposure to the QPX infected environments. The effect of ambient environmental temperature is not known at this time but is suspected to play a role in the progression of the disease. While lateral transmission from an infected bed to an adjacent uninfected bed has been observed, suggesting that QPX is a directly infective organism, transmission from the infected harbors to adjacent uninfected harbors has not yet been observed.

DNA VARIATION IN THE BAY SCALLOP, *ARGOPECTEN IRRADIANS*, IN THE WESTPORT RIVER ESTUARY, MASSACHUSETTS. Kenneth Leonard and Rocco Iocco, Jr., Water Works Group, P.O. Box 197, Westport Point, MA 02791; Thomas Sorger, Department of Biology, Roger Williams University, Bristol, RI 02809.

We assessed genetic variation among *Argopecten irradians* populations from three locations in the Westport River estuary in Westport, MA. Beginning in June, 1996, bay scallops were collected from Horseneck Channel, Jug Rock, and from a Bay Scallop

Restoration Project (BSRP) spawning raft at Corey's Island. Three scallops were collected from each location and frozen. A proteinase K digestion was performed on each sample, followed by a phenol/chloroform extraction and ethanol precipitation to isolate the DNA. The DNA was then amplified using both a specific actin primer and a short random primer in a polymerase chain reaction. The results were analyzed using agarose gel electrophoresis. It was determined that the bay scallops from Jug Rock and the spawning raft at Corey's Island lack a DNA marker found in the samples from Horseneck Channel.

JUVENILE OYSTER DISEASE (JOD)—THE VIDEO. Earl J. Lewis and C. Austin Farley, USDOC, NOAA, National Marine Fisheries Service, Southeast Fisheries Science Center, Cooperative Oxford Laboratory, Environmental Health Division, 904 S. Morris St., Oxford, MD 21654-9724.

Juvenile oyster disease has been a devastating disease for growers of cultured oysters in the northeastern region of the United States from New York to Maine. A video has been prepared that presents in a preliminary format for critique: a history of the disease, effects on the cultured oyster industry, how to recognize the disease, and management techniques to avoid heavy losses of growing oysters in JOD-infected waters. The documentary is based on interviews of affected growers from New York, Rhode Island, and Maine as well as results of scientific investigations. The video is expected to be a useful education tool for oyster growers, shellfish managers, extension agents, and for teaching students involved in shellfish or marine curricula.

AN OVERVIEW OF THE ENDANGERED SPECIES ACT AND ITS IMPLICATIONS FOR AQUACULTURE IN NEW ENGLAND. Laurie Silva, USDOC, NOAA, National Marine Fisheries Service, Habitat and Protected Resources Division, One Blackburn Drive, Gloucester, MA 01930.

The National Marine Fisheries Service (NMFS) has jurisdiction over most marine species listed under the Endangered Species Act of 1973, as amended (ESA), which includes marine mammals, sea turtles, and shortnose sturgeon. These species seasonally occupy a coastal and offshore habitat that stretches from the Gulf of Mexico to the Gulf of Maine. They live in an often hazardous environment with a variety of human-induced impacts. Certain habitats, such as concentration areas, migratory routes, and areas for breeding, reproduction, and foraging are more critical to the survival of these species than others. Potential impacts from aquaculture operations range from direct injury and mortality from lines and cables to more subtle impacts on the food chain. The species, location and critical components of habitats and the potential impacts from culture operations will be identified.

The development of aquaculture represents a change in marine resource harvesting methods from a system of hunting/gathering to

farming/culturing. Historical methods of harvesting resources from the sea are not without conflicts with endangered and threatened species of marine life, and the new methods and requirements of farming in the oceans come with a whole new variety of gear types and methods. The ESA requires federal agencies to enter into consultation with NMFS on any activities in the marine environment that may interact with protected species and determine what reasonable and prudent measures or alternatives can be implemented to allow the activity to continue without undue harm to the species. In addition to giving an overview of the species, this discussion will review the requirements under the Act as it relates to the Aquaculture permit process. Understanding how to identify the potential impacts and the requirements of the law in the early planning stages of new aquaculture ventures will help reduce the burden of the ESA portion of the federal review process.

AN OVERVIEW OF THE FEDERAL REVIEWING PROCESS FOR AQUACULTURE PROJECTS IN THE NORTHEAST REGION AND RECOMMENDATIONS FOR STREAMLINING THE PERMITTING PROCESS. Michael Ludwig, USDOC, NMFS, Habitat and Protected Resources Division, Milford, CT 06460.

The National Marine Fisheries Service has recently developed National and Regional planning documents regarding aquaculture. The two efforts have caused a number of concerns, particularly regarding the term "environmentally compatible aquaculture." Both documents were drafted to recognize the Agency's objectives that aquaculture efforts be given every opportunity to succeed while satisfying the federal laws regarding the use of Public Trust Resources. Because NMFS, as well as its sister reviewing agencies, has found it necessary to often request additional information regarding aquaculture proposals, it is taken that we are opposed to the proposal. This is not correct. Frequently, permit delays are related to lack of site-specific information, not the mechanics of the regulatory process.

To overcome these problems we have sought and created regulatory guidance to assist in the permitting process in Maine, Massachusetts, and New York. These efforts, some of which are under development, are intended to: 1) identify the types of conditions that provide confidence that an aquaculture activity will be "environmentally compatible" and 2) address potentially conflicting objectives. Our success with that guidance has led us to conclude that the effort merits expansion throughout the Northeast. To that end, we are in the process of forming an outreach group that will work with state and federal regulators in drafting straightforward guidance on siting criteria and system monitoring packages that should streamline regulatory reviews. However, the guidance will not rescue projects that are not environmentally compatible.

SIROLPIDIUM ZOOPHTHORUM, LETHAL FUNGUS PARASITE OF BIVALVE LARVAE: RECENT OBSERVATIONS IN BAY SCALLOP CULTURES. Christopher Martin, Sheila Stiles, Joseph Choromanski, and James C. Widman, Jr., USDOC, NOAA, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, Milford, CT 06460; Daniel Schweitzer, University of Connecticut, Marine Sciences and Technology Center, Groton, CT 06430; Christopher Cooper, Sea Change Foundation, Covington, VA 24426.

A simple holocarpic marine phycomycete has been observed in cultures of larval bay scallops at the Milford Laboratory. The parasite appears to be identical to one first reported by Victor Loosanoff over 40 years ago in larvae of several bivalve species. Named *Sirolpidium zoophthorum* by H. S. Vishniac in 1955, it belongs to a small group of primitive marine fungi in the order Lagenidiales. The parasite develops within the soft tissues of the larvae eventually absorbing the entire body. Fully developed thalli of the fungus appear as spherical bodies or sparsely branched tubes. The highly vacuolated multinucleate cytoplasm of mature thalli produce biflagellate zoospores by progressive cleavage. At first, these vigorously swimming cells can be seen actively milling about in the parent cell, later emerging through specialized exit tubes. Infection of healthy bivalve larvae has not been directly observed, but comparison with events recorded for other invertebrate hosts suggests that infection is initiated by the attachment of one or more zoospore to the body wall. So far, there is no evidence of ingestion of the zoospores as a route of infection. Resistance to the parasite is unknown. Infected larvae are doomed. The importance of this parasite in the health and survival of hatchery-spawned bay scallops is discussed.

EXPERIENCES IN AQUACULTURE: THE GOOD, THE BAD, AND ISSUES YET TO BE RESOLVED. Harold C. Mears, USDOC, NOAA, National Marine Fisheries Service, One Blackburn Drive, Gloucester, MA 01930.

In the northeastern United States, there has been a dramatic increase in federal funding support for aquaculture research and development during the last three years. Activities supported by grant programs administered by the National Marine Fisheries Service have emphasized cost-effective approaches for advancing environmentally-sound private aquaculture development, feasibility of aquaculture as an enhancement tool for rebuilding overexploited finfish and shellfish populations, and its potential in providing new business opportunities for displaced fishermen.

This emphasis is succeeding in building new partnerships among the public, private, and academic sectors and has begun to answer important questions concerning culture technology, system design, and economic viability. Recent experiences have further focused attention on the time-consuming logistics of permitting requirements for aquaculture operations at the local, state, and

federal levels. Activities are also addressing the implications of known and potential environmental impacts and market uncertainties.

Increased financial support for aquaculture is serving as a catalyst for dealing with technical, political, and managerial concerns which otherwise would have surfaced over a more extended horizon. The ultimate success in their resolution will be dependent upon the further assessment, likely from a geo-political perspective, of aquaculture as it relates to sustainable economic development and resource stewardship responsibilities among local, state, and federal jurisdictions. The prognosis continues to be one of guarded optimism.

TAUTOG CULTURE: PRELIMINARY STUDIES. **Renée Mercaldo-Allen, Dean M. Perry, Catherine Kuropat, and James Hughes,** USDOC, NOAA, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, Milford, CT 06460.

Preliminary studies have been conducted to establish a culture protocol for the blackfish or tautog, *Tautoga onitis*. Tautog are a popular recreational species valued for their mild flavor and white-flaked texture. An Asian seafood market exists for 1 pound or "wok sized" fish. Depleted natural stocks could potentially be enhanced by releasing cultured fish back into the environment. Studies over the last two years at the Milford Laboratory have resulted in culture of tautog to 1.5 years old and suggest that tautog aquaculture may be feasible.

Adult tautog were allowed to spawn naturally in long raceway tanks. Eggs were collected and placed in 250-micron mesh bags held in a Styrofoam float in flowing, ambient-temperature seawater where they hatched within 48 hours. Yolk-sac larvae were transferred to green-water culture tanks which were stocked with protozoans and rotifers for the tautog to prey upon. These prey items were fed an alga high in fatty acid content to provide an enriched high quality food for the tautog. Brine shrimp nauplii, and eventually adults, were introduced later to provide larger size prey. Daily monitoring of tank conditions indicated that static culture beyond a few weeks duration resulted in elevated ammonia concentrations and reduced water quality. Therefore, flowing seawater was added to the tanks at a slow rate once the yolk-sac was resorbed, approximately 6 days post-hatch. At this time, the fish had acquired pigment and were observed actively feeding in the water column. Slow flow-through green-water culture kept concentrations of ammonia and other wastes in the tanks at low levels, but allowed food items to be retained long enough to allow adequate feeding. Tautog larvae proved to be very sensitive to handling for the first month or two post-hatch.

When ambient water temperatures declined, three-month-old cultured tautog were transferred to recirculating seawater tanks at room temperature to promote year-round growth. Fish were weaned onto an artificial diet, supplemented with chopped mussel and clam. The goal for potential aquaculture of tautog is growth

from larvae to 1 pound or "wok" size within a two-year period. Work at the Milford Laboratory continues to refine culture methods to determine whether tautog would be a good candidate for finfish aquaculture and enhancement of wild stocks.

ADVENTURES IN LOW SALINITY OYSTER CULTURE: STRATEGIES FOR COPING WITH TOO MUCH FRESH WATER. **Donald W. Meritt and Garry J. Baptist,** University of Maryland Horn Point Environmental Laboratory, 2020 Horn Point Road, Cambridge, MD 21613; **Jacqueline U. Takacs,** University of Maryland Sea Grant Extension Program, Chesapeake Biological Laboratory, P.O. Box 38, Solomons, MD 20688; **Kennedy T. Paynter,** University of Maryland Department of Zoology, College Park, MD 20742; **Robert M. Pfeiffer,** Maryland Oyster Recovery Partnership, P.O. Box 6776, Annapolis, MD 21401.

Maryland oyster populations are currently experiencing all time low levels of production. Problems stemming from long-term overharvest coupled with recent high levels of disease have caused harvests to plummet. As a result, the Maryland Department of Natural Resources convened the Maryland Oyster Roundtable (MOR) in 1993. The forty-member MOR formulated an action plan for oyster recovery in Maryland. One important aspect of this plan was the establishment of Oyster Recovery Areas or ORAs.

Activities within various ORA designations have been restricted. ORAs have been classified into three categories, A, B, and C. Only oysters that have tested negative for disease may be introduced into any zone A or B, and there will be no harvest allowed within zone A. Zone C has no restrictions on oyster movement and commercial harvest is allowed in both zones B and C.

Studies have been designed to determine the feasibility of growing oysters within these zones by using non-diseased oyster seed produced in hatcheries. During 1994, a ten-acre area of oyster growing bottom was set aside in zone A in the Upper Choptank River and planted with over 100,000 bushels of dredged oyster shell. This test plot was divided into two five-acre sub-plots for planting. During 1995, 2.5 million oyster seed produced by the Horn Point Hatchery were deployed on the first test plot. In 1996, 5 million additional oyster seed were deployed on the second sub-plot.

Data have been collected on setting efficiencies, survival, growth, and disease prevalence on all batches of oyster seed used on these test plots. Additionally, continuous water quality measurements have been collected using a data sonde.

The hatchery seasons of 1995 and 1996 differed dramatically. Record snowfalls and a very wet spring and summer produced some of the lowest salinities on record for the upper Chesapeake Bay during the 1996 hatchery season. Salinities that had remained fairly high during 1995 (10–13 ppt) dropped below 8 ppt for the duration of the spawning season at the HPEL hatchery. This delayed normal spawning activity until August. Oyster broodstock in the river also remained ripe well past the normal spawning period.

Efforts were made to artificially raise hatchery salinities to

allow for production to commence. Broodstock were spawned and larval rearing was attempted at very low ambient salinities. Subsequent efforts were conducted using sea salts, and finally by using a mixture of ocean water and Choptank River water. There were no larvae successfully reared using ambient Choptank River water, and only limited success with the addition of salt; however, the mixture of ocean water with Choptank River water was successful.

Spawning, larval rearing, and settlement proceeded normally once ocean water was mixed with ambient river water in the hatchery. At two days post settlement, spat were gradually acclimated down to ambient salinity conditions and survival and growth were normal. Nursery areas were selected based on salinity.

Oyster seed exhibited excellent growth during the nursery period for both years although some of those produced in 1996 were removed at a smaller size than the previous year. Factors contributing to this decision were water temperature and infestation by *Stylococcus elipticus*.

Survival of both groups of seed on the test plots has been excellent given the conditions present during the summer of 1996 when salinities remained below 4 ppt for over 4 months and occasionally remained below 2 ppt and sometimes less than 1 ppt for varying periods of time. Spat deployed during 1995 showed 60% survival for the test period, and spat deployed during 1996 have shown little mortality although they have not been deployed long enough to provide meaningful data.

Perkinsus marinus was found in one spat during the last testing (at time of deployment in 1995), and a later sampling revealed two spat tested positive for Perkinsus. Since the winter of 1995/96 no oyster spat have tested positive for disease either in the nursery areas or on the test plots.

Funding for this study was provided by NOAA, Sea Grant, Maryland Department of Natural Resources, and the Maryland Oyster Recovery Partnership. Much of the labor for moving shell bags was provided by the Living Classrooms Foundation and Cambridge South Dorchester High School and many volunteers whose assistance was invaluable in moving the large amounts of materials needed.

GREEN-WATER CULTURE OF TAUTOG. Dean M. Perry, Renée Mercaldo-Allen, Catherine Kuropat, and James Hughes, USDOC, NOAA, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, Milford, CT 06460.

Tautog (*Tautoga onitis*) embryos were cultured to hatching and raised successfully through the difficult larval stage to juveniles using green-water culture. Spawning of field-collected adult tautog was accomplished under laboratory conditions. Tautog allowed to spawn naturally produced more viable embryos than those spawned artificially.

Eggs were collected and placed in 250-micron mesh bags held in flowing-ambient-temperature seawater where hatching occurred within 48 hours. Yolk-sac larvae were transferred to round 4 ft

tanks of static sand-filtered seawater where they were fed protozoans for 4–6 days post-hatch, followed by rotifers from days 2 to 20 post-hatch and brine shrimp from day 7 to several months post-hatch. *Utex*, an alga high in fatty acid content, was added to the tanks to enrich the prey items, hence the term green-water culture. Slow flow-through green-water culture proved superior to static methods of culture. Flowing water prevented build-up of waste products and maintained high water quality. The young larvae were very sensitive and handling resulted in high larval mortality.

Tautog were cultured in the green-water tanks until ambient seawater temperatures began to decline. The fish were moved indoors to recirculating seawater tanks at 3-months old and were weaned onto a diet of chopped mussel, clam, and a commercial food.

ATACOSPA AQUACULTURE PROJECT—SUBTIDAL GROWOUT OF QUAHOGS. Bruce A. Peters, Atacospa Aquaculture, P.O. Box 947, East Orleans, MA 02643.

The Atacospa Aquaculture Project is an exploration into subtidal hard clam growout techniques. The continued development of our increasingly populated shorelines demands that we research alternate methods to bivalve aquaculture that minimize both user conflicts and visual impacts. The Atacospa Aquaculture Project intends to show the benefits of the subtidal methods. Although every area has its differences, the information provided by the project will open doors to other projects similar in design.

The project will provide the information learned in a guidebook, or how-to manual format with over twenty-four black and white photos inserted into the text. These manuals are to be distributed by both the Massachusetts Aquaculture Association and the Barnstable County, MA Cooperative Extension offices. In addition, a small slide presentation is available (<100 color slides) to accompany the discussion and presentation of the information. The project will cover all aspects of the details of subtidal growout, from site selection to the harvest of the mature animals.

The information I have provided with the Atacospa Aquaculture Project will provide an alternative to the ice and winter related damages that occur regularly on intertidal bivalve growout sites. By reducing the amount of winter related damages to the crop and gear, we can minimize the amounts of aquaculture related debris upon the shorelines. Financial institutions will become more willing to lend to the growers who show increased success rates due to their use of subtidal methods. Additional benefits include the effective removal of upland owner control to intertidal sites which are now starting to be confronted with litigation challenging existing uses of the tidelands.

Funding for this project made possible by a grant from the Massachusetts Department of Food and Agriculture

THE ASIATIC CLAM (*CORBICULA FLUMINEA*) AND WATER POLLUTANTS. Harriette L. Phelps, Department of Biological and Environmental Sciences, University of the District of Columbia, 4200 Connecticut Ave. NW, Washington, DC 20008.

The Asiatic clam (*Corbicula fluminea*) invaded the freshwater tidal Potomac River estuary near Washington, DC in 1978 and by 1984 the population of five km below DC was estimated at 8×10^6 kg. *Corbicula* has a high filtration rate and was estimated to filter from one-third to most of the water passing through that region of the estuary. It has invaded most US states, but is raised in culture in Asia and could become an aquaculture species of interest to Asians. The ability of the clam to remove the pollutants phosphate, nitrate, and iron (FeCl_3) from the water column was studied using suspensions of cultured algae (*Thalassiosira weissflogii*), mud sediment (74 u) and plankton collected from the Potomac and Anacostia rivers, and the C&O Canal. The native plankton samples had quartz fragments with some algae and organic material fragments. Suspensions were made with and without pollutants, and with and without added clams. The suspensions were at an ecologically relevant level (100 mg/l), agitated to maintain suspension, and subsamples taken over three hours. Subsamples were centrifuged and analyzed for pollutant concentration remaining in the water column. All experiments were run in triplicate.

Nitrate concentrations were not affected with or without algae, sediment, plankton, clams, or any combination of those factors. Phosphate concentrations increased in algae suspensions alone and with clams present (probably due to cell damage) but did not change in plankton suspensions with or without clams. Phosphate concentrations decreased in all sediment suspensions and much more rapidly with clams present. Iron concentrations decreased with clams present in suspensions of river plankton but not sediment or algae suspensions. Iron concentrations also decreased with clams without suspensions: mucus production was observed and may have been a factor. In conclusion, when an added water pollutant such as phosphate decreased over time, it was probably due to sorption by suspended material and settling. The removal rate was 50% higher in the presence of clams. All native plankton samples from the Anacostia, Potomac, or C&O Canal showed failure to sorb phosphate or nitrate but had strong iron sorption. This was different from suspensions of fine sediment collected from the same region which showed phosphate sorption but no iron or nitrate sorption. Algae suspensions released phosphate and did not sorb nitrate or iron. These differences from suspensions of natural planktonic material suggest that surrogate suspended materials (cultured algae and sediment) might not be valid in predicting pollutant concentrations in natural freshwater systems such as rivers and canals. The sorptive ability of native suspended material, which controls the water column concentrations of added pollutants, is probably due to its organic surface material composed of bacterial layers typical of the salinity and season. The selective translocation of water column pollutants to the benthos is greatly aided by the rapid filtration action of the Asiatic clam as it forms pseudofeces.

A LOW-COST FLOATING AXIAL-FLOW UPWELLER SHELLFISH NURSERY SYSTEM. Gregg Rivara, Cornell Cooperative Extension-Suffolk County Marine Program, 3690 Cedar Beach Road, Southold, NY 11971; David Bavaro, Shellfish Construction and Culture Company, 67 Hill Street, Wading River, NY 11792.

Nursery culture of shellfish is often thought of as the bottleneck to shellfish production; many hatcheries supply small seed and there are many acres of underwater land for growout. Growing the shellfish from the hatchery to a field-plantable size is more difficult, due to the costs associated with traditional land-based nurseries. These costs include construction capital, waterfront land, electricity, and labor. Nursery systems that float on the culture water require less electricity and can be placed in marinas or other areas at a much lower cost than traditional tank and silo systems.

The Cornell axial-flow shellfish nursery system uses current upweller technology coupled with a simple and low cost axial-flow pump. The combination of the efficient pump and the very low head of the floating system allows the system to run quietly with a much lower operating cost than traditional land-based centrifugally-pumped systems. Economy is enhanced by the large silo size used in the system. Construction techniques, operation, and maintenance of a prototype system will be discussed.

DESIGN AND RESEARCH PLAN FOR THE MILFORD PHYTOPLANKTON CULTURE GREENHOUSE FACILITY. Barry C. Smith and Gary H. Wikfors, USDOC, NOAA, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, Milford, CT 06460.

The newly constructed greenhouse at the Milford Laboratory is designed to allow experimental investigations of algal culture on both a production scale and in scale-up. The building itself is 30' \times 32' with a galvanized pipe frame and a transparent covering. There are two 4,700 gallon (17,150 liter) oval culture tanks placed two feet into the gravel floor and eight 137 gallon (500 liter) cylindrical culture tubes along the north wall. Solar illumination can be supplemented with twelve 1,000 W sodium vapor lamps suspended over the oval tanks and fluorescent fixtures behind the cylindrical tanks. Underground pipe-chases connect the greenhouse to the laboratory's "tank farm" building housing animals to be fed. Maximum production should be able to exceed 5,000 gallons (18,250 liters) per day of dense algal suspension.

The research effort in this facility will focus upon the application of industrial process-control technology to phytoplankton culture for aquaculture feeds. We have designed and are assembling computer-controlled, automated systems to minimize labor while increasing flexibility and accuracy. Ultimately this research will allow increased production and reduce the cost of large-scale algal culture.

EARLY RESPONSES TO SELECTION FOR GROWTH IN THE BAY SCALLOP, *ARGOPECTEN IRRADIANS*, FROM LONG ISLAND SOUND. Sheila Stiles and Joseph Choromanski, USDOC, NOAA, National Marine Fisheries Service, Milford Laboratory, Milford, CT 06460; Daniel Schweitzer, Marine Sciences and Technology Center, University of Connecticut, Groton, CT 06340.

Precipitous declines in natural populations of economically valuable aquatic species as scallops, concomitant with an increase in their imports contributing to trade deficits, have led to heightened interest in aquaculture. Moreover, success achieved with the selective breeding of oysters from Long Island Sound for fast growth has increased the expectation that other shellfish, in particular scallops, can similarly be improved.

To investigate heritable responses to selection for growth of bay scallops (*Argopecten irradians*) from Long Island Sound, wild native bay scallops from Stonington, Connecticut were mass-spawned in 1995 to establish four genetic lines for selective breeding. Progeny were cultured and grown to maturity. The following year, the fastest growers or largest animals were selected and spawned to produce a subsequent generation. Concurrent selection was performed for the slowest growers or smallest specimens as controls for determining whether differences in size between groups could be attributed to genetic selection. Variations in early growth responses were observed among the first selected generation lines of scallops. Explanations for these results are discussed.

Partial funding for this research was provided by the Marine Sciences and Technology Center of the University of Connecticut.

MAKING THE PERFECT SPAT BAG FOR COLLECTION OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS*.

Karin A. Tammi, Eric Buhle, and Wayne H. Turner, The Water Works Group, Inc., P.O. Box 197, Westport Point, MA 02791; Victor Satkin, SeaTech Corp., Division of Satkin Industries in New Bedford, MA 02740.

Since its inception in 1993, The Bay Scallop Restoration Project (BSRP) has been re-establishing bay scallop, *Argopecten irradians*, populations in the waters of the Westport River, Massachusetts. Enhancement of the scallop stocks has been achieved by the establishment of spawning sanctuaries and the deployment of artificial spat collectors throughout the estuary. Observations from studies conducted since 1994 indicate that poor scallop recruitment in artificial spat collectors can be attributed to crab predation, fouling, and surface area of the settlement substrate inside the collectors. As a result, researchers have been investigating methods to improve scallop recruitment to artificial collectors not only by studying the spawning and settlement time of scallops, but also by perfecting the design of the spat collector. In 1995, researchers determined that a commercial fine-mesh collector containing a polyethylene tube as the settlement substrate performed significantly better than an onion-bag collector stuffed with monofilament. Conclusions indicated that the fine-mesh collector exhibited

a greater surface area for settlement and did not allow mud crab colonization inside the collector. Although this collector performed well, its durability was a concern and was cost prohibitive for the BSRP to use. Thus, in 1996 researchers developed a new spat collector made locally by the SeaTech Corp., a Division of SATKIN Industries in New Bedford. The performance of this new collector dubbed the SPATKIN bag was compared with the onion-bag and fine-mesh collectors containing a combination of monofilament and polyethylene tubing as the settlement substrate. Two longlines consisting of 20 collectors, 10 of each bag type with similar stuffing, were deployed at three study sites soaking for a period of 30 and one longline until 100 days. After soaking, bags were harvested to assess fouling, crab abundance, and scallop recruitment in each type. In general, those longlines in the water for 30 days showed that the SPATKIN bag did not display significantly higher scallop recruitment than the other bag types. However, longlines soaking 100 days showed that the SPATKIN bag significantly displayed higher scallop recruitment ranging from ($p < 0.05$) to ($p < 0.001$) out performing the other two bag types at all three study sites. The SPATKIN bag collected a total of 717 scallops compared to 168 from the fine-mesh collector and exhibited greater recruitment estimates averaging 88 scallops per collector compared to 21 scallops per fine-mesh collector. Both bag types were stuffed with 400 grams of monofilament. Secondly, SPATKIN bags significantly ($p < 0.01$) to ($p < 0.001$) out performed fine-mesh collectors when polyethylene tube was inserted as the settlement substrate, collecting a total of 454 scallops compared to 182 in the fine-mesh collector, averaging 50 scallops per collector compared to the fine-mesh collector which caught 20 scallops per collector. Lastly, the SPATKIN bag significantly ($p < 0.05$) to ($p < 0.001$) out performed the onion-bag collector; both stuffed with 400 grams of monofilament, collecting a total of 754 scallops compared to 40 from the onion bag. The SPATKIN bag averaged 84 scallops per collector compared to only 4.4 scallops per onion-bag collector. Although monofilament stuffed collectors appear to display higher scallop recruitment, performance could not be clearly demonstrated. Research from the summer of 1996 indicates that the SPATKIN bag performs well as a spat collector for bay scallops, *Argopecten irradians*. Furthermore, the SPATKIN bag was more durable, prevented mud crab predation compared to the other collector types tested, and was very cost effective for the BSRP. This collector shows promise for future use in other estuaries and for hatchery settings.

NEW PICK-UP TRUCKS INVADE WESTPORT! THE DEVELOPMENT OF A SHELLFISH MARKETING COOPERATIVE. Wayne H. Turner, The Water Works Group, Inc., P.O. Box 197, Westport Point, MA 02791.

In an effort to demonstrate the economic value of the bay scallop to southeastern Massachusetts, The Water Works Group has paired its progress in shellfish propagation and resource man-

agement with the marketing experience of the member-owned Coastal Growers Association (CGA). The result has been the establishment of the first farm produce/seafood marketing cooperative in Massachusetts.

To appreciate the magnitude and significance of this event requires an understanding of the history of a community's determination to make shellfishing an economic and an environmental reality. In 1993, the Bay Scallop Restoration Project (BSRP) was launched by the nonprofit Water Works Group as a positive step towards improving water quality in the Westport River. Since its inception, the strategies of the BSRP have led to the reopening of 70% of the Westport River to shellfishing. Successful propagation programs coupled with the improved water quality have generated the first major bay scallop harvest in Westport since 1985.

In October 1996, twenty-one commercial fishermen joined CGA committing 100% of their catch to CGA. These fishermen are also investing 3.5% of their profits in the sustainable shellfish propagation projects led by The Water Works Group. As a result of the banner bay scallop crop in the Westport River, these twenty-one fishermen harvested 25 tons of shellstock, contracted with several local shucking facilities, and a contract individual quick freezing company (IQF Custom Packing). CGA has developed wholesale and retail packaging as well as a trade name: "Heritage Farm Coast Bay Scallops" promoting the Heritage Farm Coast region which includes Buzzards Bay and Narragansett Bay.

Historically in Westport, banner bay scallop seasons are remembered by the purchase of new pick-up trucks. As a result of the 1996 harvest, almost all 1985 vintage trucks are being retired and replaced with 1997 models. The combination of successful bay scallop propagation strategies coupled with innovative marketing programs like the cooperative model are shedding light onto opportunities with other shellfish and seafood products. Employment considerations make this a significant event as one hundred twenty-five (125) new jobs were created in the region. Though the harvest season lasted only seven weeks, long-lasting economic opportunities in job creation, income, food, private investment opportunities, and the building of a constituency financially vested in the improvement and maintenance of water quality have been brought to the forefront of public interest.

PHYTOPLANKTON CULTURE FOR NURSERY REARING OF POST-SET BIVALVES: SCALING EXERCISES OR WE CAN'T AFFORD TO DO THAT! CAN WE? Gary H. Wikfors, USDOC, NOAA, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, Milford, CT 06460; Loy Wilkinson, Coastal BioMarine, Bridgewater, CT 06430.

Most molluscan hatcheries feed larvae selected phytoplankton strains that are cultured on site. Following metamorphosis, post-set animals may be fed cultured algae for an additional period of days

to several weeks. Thereafter, post-set are moved to land- or sea-based nursery systems in which the only food source is natural phytoplankton. Continuing to feed cultured algae to post-set bivalves is considered not to be economically viable, even though natural phytoplankton has several disadvantages (food quantity or quality may be poor and animals may be exposed to disease or predators at a susceptible size), and nutritionally-superior post-set algal diets have been found. Indeed, with algal-culture production costs on the order of \$300–\$500 per dry kilogram of algal biomass at most small- to medium-sized hatcheries, the economics of feeding cultured algae to post-set bivalves are not encouraging using current methods.

Here we ask the questions: "Why is algal biomass so expensive to produce?" and "What can be done to bring the cost of algal culture down?" Biological limitations of most current algal-culture systems involve two processes: light utilization and gas exchange. Self-shading by dense cultures in most bottles and tanks (with three approximately equal dimensions) results in light-limited growth rates, often leading hatchery operators to increase artificial lighting at considerable expense. Gas-exchange problems can involve: 1) the inability of air-bubbling to provide enough carbon dioxide to keep pace with photosynthesis in open systems, leading to inhibitory pH increases, or 2) build-up of diatomic oxygen in closed systems, such as tubular designs, leading to photosynthetic inhibition. Innovative designs of algal culture apparatus are needed that address these biological limitations.

A first-order economic analysis of current algal-culture systems shows that greatest economic benefits will result from addressing four areas: 1) use of natural light, 2) high cell density to maximize light utilization and permit use of small culture vessels, 3) a continuous process to minimize labor, and 4) sterile operation to minimize "crashes" and increase shelf-life. Accordingly, development of a continuous, automated process designed around efficient utilization of natural sunlight, and incorporating effective gas exchange, offers the most attractive economic benefit for improving algal culture technology for nursery feeding of bivalves.

DOMESTICATION OF *PORPHYRA* (=NORI) FOR NORTH-EAST AMERICA. Charles Yarish, Gretchen Frankenstein, and Alexis E. Sperr, Department of Ecology and Evolutionary Biology, University of Connecticut, Stamford, CT 06903; Xingeng C. Fei, Experimental Marine Biology Laboratory, Chinese Academy of Sciences, Qingdao, People's Republic of China; Arthur C. Mathieson, Jackson Estuarine Laboratory, University of New Hampshire, Durham, NH 03824; Ira Levine, Coastal Plantations International, Inc., Poland, ME 04274.

A variety of field and culture studies are being made in order to clarify the taxonomic status, ecological requirements for enhancing the mariculture potential of several *Porphyra* species from coastal New England. At least six different species of *Porphyra* are

being examined using a variety of traditional morphometric and cytological parameters. Detailed seasonal and spatial collections, from diverse coastal and estuarine habitats, are being used to delineate the seasonality and habitat preferences of *Porphyra* in northeast New England. Unialgal cultures of *Porphyra amplissima* (Kjellman) Setchell & Hus in Hus, *P. miniata* (C. Agardh) C. Agardh, *P. umbilicalis* (Linnaeus) J. Agardh, *P. linearis* Greville, *P. purpurea* (Roth) C. Agardh, and *P. leucosticta* Thuret in Le

Jolis have been established and are being maintained for comparative molecular genetic and physiological investigations. Several strains of *P. amplissima*, from coastal Maine, have successfully completed their life cycles in culture and F₂ individuals have been obtained. Strains of this taxa are now being transferred to shell culture for field trials in the spring. A discussion of integrating native northeast America *Porphyra* species within the existing commercial nori farm will be presented.

ABSTRACTS OF TECHNICAL PAPERS

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BIVALVE BIOLOGY

EFFECT OF LIPID SUPPLEMENTATION DURING BROODSTOCK CONDITIONING OF MARINE BIVALVES. Marrit Caers,* Peter Conttean, and Patrick Sorgeloos, Laboratory of Aquaculture and Artemia Reference Center, University of Ghent, Rozier 44, B-9000 Gent, Belgium.

It is known that lipids play an important role during the maturation process in marine bivalves. The present study investigated the possible use of emulsions as a carrier to supplement algal diets with essential fatty acids during broodstock conditioning of *Argopecten purpuratus* and *Crassostrea gigas*. The uptake and assimilation of the emulsions were verified analytically by fatty acid and lipid analysis of broodstock, eggs and larvae. *A. purpuratus* was fed a mixed algal diet of *I. galbana* (T-Iso), *P. lutheri*, *C. calcitrans* and *D. tertiolecta* (1:1:1:1 on DW basis) whether or not supplemented with an emulsion rich in 22:6n-3 (DHA) or 20:5n-3 (EPA). The objective was to collect information concerning the effect of quality and quantity of dietary lipids on fecundity, egg composition and anatomical distribution of lipids and fatty acids in selected tissues of the scallop (gills, mantle, adductor, digestive gland, male and female gonad). *C. gigas* was fed a mixed algal diet of *Isochrysis*, *Dunaliella* and *Rhodomonas* (1:1:1 on DW basis) or a monospecific algal diet of *Dunaliella tertiolecta*. To evaluate the importance of essential fatty acids, *D. tertiolecta*, which lacks highly unsaturated fatty acids (HUFA), was fed with and without an emulsion rich in (n-3) HUFA, mainly DHA. The aim was to determine the influence of dietary lipids on the lipid and fatty acid composition of eggs and larvae. Furthermore, it was investigated if the lipid or fatty acid composition could be correlated with the success of embryonic and larval development. Finally, the fecundity and quality of eggs and larvae were compared with those of the oysters fed the mixed algal diet which was known to have a good nutritional value for the reproductive conditioning of *C. gigas*.

RELATIONSHIP BETWEEN LARVAL AND JUVENILE GROWTH RATES IN THE HARD CLAM *MERCENARIA MERCENARIA*. Carrie J. Deming* and Michael P. Russell, Department of Biology, Villanova University, Villanova, PA 19085.

Hard clam aquaculture practices select for fast-growing seed by culling out the small individuals during early larval stages. One goal of culling procedures, or larval size selection, is to maximize industry efficiency by producing clams that reach market size in the least amount of time. However, there is little evidence to support the assumption of a positive relationship between larval and juvenile growth. We quantitatively evaluated the current practice of larval culling using two approaches—an unreplicated experiment in a hatchery and a companion replicated experiment in the

laboratory. Larvae raised from a mass spawn at Biosphere Inc. (a hatchery in NJ) were separated into two different size classes (small and large) to test the implicit assumption that large larvae develop into marketable adults more quickly. We followed standard industry procedures (except larval culling) and measured shell lengths over a period of six months. A repeated-measures ANOVA was used to test the null hypothesis that there were no differences in juvenile growth due to larval size selection. Growth rates of the small and large larval groups in the small-scale laboratory experiment have paralleled those in the industrial-scale setting at Biosphere. At the onset, the size difference between the two groups was significant. The difference in shell length eventually disappeared and the two groups became similar in size in both the laboratory and the field. We have demonstrated that the prevailing assumption in the industry of a positive growth relationship between larvae and juveniles is false.

LONG-TERM PATTERNS OF OYSTER SETTLEMENT IN A RELATIVELY UNDISTURBED, HIGH SALINITY SOUTH CAROLINA ESTUARY. Paul D. Kenny, Dennis M. Allen, and David Bushek, Baruch Marine Field Laboratory, University of South Carolina, Georgetown, SC 29442.

The settlement patterns for the eastern oyster, *C. virginica* have been studied since 1982 in a high salinity southeastern estuary where oyster form densely populated intertidal reefs. Vertical arrays (three levels) of collecting plates were deployed for consecutive two week intervals from May to November at one site and examined for oyster spat—a previous study demonstrated that factors controlling oyster settlement in this estuary are operating at the ecosystem or broader spatial scale. Within year fluctuations in abundance were large, but early and late season peaks usually occurred. Within and among year differences in settlement timing and intensity were generally not related to changes in water temperature and salinity, but low recruitment generally coincided with extreme conditions. Variations in other system-wide factors affecting behavior and survival of larvae and newly settled spat are probably more important in controlling intra- and interannual patterns of oyster settlement during average years. Gregarious settlement and competition with other invertebrates for space indicate that biological interactions are important determinants of settlement and early recruitment.

UTILIZATION OF CARBON FROM THE MICROPHYTOBENTHOS BY THE RIBBED MUSSEL, *GEUKENSIA DEMISSA*. Daniel A. Kreeger,* Patrick Center for Environmental Research, Academy of Natural Sciences, Philadelphia, PA 19103; Roger I. E. Newell and Shou-Chung Huang, Horn Point Environmental Laboratory, University of Maryland, Cambridge, MD 21613.

In salt marshes, metazoan consumers such as suspension-feeding bivalves ingest a complex suite of particles from the

seston. To augment our ongoing research on whether sources of organic C other than phytoplankton can contribute to the nutritional requirements of the ribbed mussel, *Geukensia demissa*, we measured this species' ability to ingest and digest C from dominant species of microphytobenthos. Four species of benthic diatoms and one species of cyanobacteria were isolated from the marsh surface in New Jersey and Delaware and put into unialgal culture. Three additional species of benthic diatoms originally isolated from salt marshes were obtained from the Bigelow Collection. These benthic microalgae were uniformly labeled with ^{14}C and fed to mussels under conditions in which algal cells were maintained in constant suspension by mixing. Ribbed mussels filtered all species of benthic microalgae at rates (0.7 to $1.4 \text{ L h}^{-1} [\text{g dry tissue weight}]^{-1}$) greater than those ($0.6 \text{ L h}^{-1} [\text{g dry tissue weight}]^{-1}$) for mussels fed *Isochrysis galbana* clone T-ISO, a planktonic unicellular alga commonly used as a food source for bivalves. All microalgae were assimilated efficiently, and there were no significant differences among species of benthic microalgae (63 to 93%) or compared with the T-ISO control (84%). Our results demonstrate that if microphytobenthos cells are suspended into the water column, they can be efficiently utilized by *G. demissa*, a keystone consumer in the intertidal zone of eastern USA salt marshes.

IMPACT OF CRYOPROTECTANTS DIMETHYL SULFOXIDE, ETHYLENE GLYCOL, METHANOL, GLYCEROL, SUCROSE AND POLYVINYLPYRROLIDONE ON OYSTER (*CRASSOSTREA GIGAS*) EMBRYOS BEFORE FREEZING. Xin Liu* and Anja M. Robinson, Department of Fisheries and Wildlife, Hatfield Marine Science Center, Oregon State University, Newport, OR 97365.

Information on impact of cryoprotectants on oyster embryos before freezing is a key important factor for successful oyster embryo cryopreservation. Oyster embryos were exposed to various concentrations of six cryoprotective compounds, dimethyl sulfoxide (DMSO), ethylene glycol (EG), methanol, glycerol, sucrose and polyvinylpyrrolidone (PVP) at room temperature ($21\text{--}24^\circ\text{C}$) for 30 minutes. The results showed that toxicity impact of the tested chemicals on oyster embryos varied with the development stages, the later trochophore stage being more resistant than the earlier two to four cell stage, and the toxicity impact on survival rates of oyster embryos increased as the tested chemical concentration increased. Glycerol was highly toxic to oyster embryos at greater than 1.5 M concentrations. Sucrose and ethylene glycol had more than 80% survival rates at less than 0.3 M and 1.8 M concentrations, respectively. PVP at less than 10% concentrations did not have toxic effects on oyster embryos. The results of time exposure experiment conducted with 1.4 M DMSO, 1.8 M EG, 2.4 M methanol, 1.4 M glycerol, 0.292 M sucrose, and 10% PVP concentrations for 60 minutes indicated that the exposure time should be less than 30 minutes to minimize the injury of the oyster

embryos caused by cryoprotectants. The combinations of 0.7 M DMSO + 0.9 M EG, 0.7 M DMSO + 1.2 M methanol, 0.7 M DMSO + 0.141 M sucrose, 0.7 M DMSO + 10% PVP, 0.9 M EG + 10% PVP, and 0.141 M sucrose + 10% PVP improved oyster embryo survival rates (20 minute exposure). It could be attributed to the replacement of each chemical fraction with the other rather than any specific toxicity blocking mechanisms. The exposure to the cryoprotectants before freezing could cause major biochemical or/and osmotic injury on oyster embryos.

THE NATURAL HISTORY AND HABITAT CHARACTERISTICS OF SOFTSHELLS (*MYA ARENARIA*) IN NORTHERN NEW JERSEY. Clyde L. MacKenzie, Jr., James J. Howard Laboratory, Northeast Fisheries Science Center, NMFS-NOAA, Highlands, NJ 07732.

The natural history and habitats of softshell clams in Raritan Bay and the Navesink and Shrewsbury Rivers, NJ, were studied from 1993–96. Settlement densities of juveniles ranged as high as $7,000/\text{m}^2$. Causes of mortality varied among beds. Juveniles that settled on impenetrable hard clay substrates did not survive. Most settled in sand sediments, fewer in mud. Within weeks after settlement, many clams emerged from the bottom (cause not identified), laid on the surface, and died in 4–6 weeks. Observed predators of clams were fishes (mainly *Fundulus* sp.), black ducks (*Anas rubripes*), and horseshoe crabs (*Limulus polyphemus*). Man-related causes of mortality were: 1) smothering under mats of sea lettuce (*Ulva lactuca*) (possibly caused by eutrophication); 2) large waves dislodging the clams from sediments (this followed the loss of eelgrass in the 1940's; eutrophication has since prevented eelgrass growth; before then, the eelgrass had dampened the effects of waves on the clams); and 3) in July–August, 1995, most clams in the two rivers died when water temperatures persisted at about $30\text{--}31^\circ\text{C}$ for several days (global warming?). In beds with no evident causes of mortality after the clams had attained a length of at least 15 mm, the survival rate was about 50% in 21 months, September 1993 to June 1995. The clams attained market size about 2 years after settlement. Disease infections in the clams are being monitored quarterly by the Oxford, MD, NMFS laboratory.

CHARACTERIZING THE RELATIONSHIP BETWEEN *CRASSOSTREA VIRGINICA* AND A HYDROZOAN INQUILINE SYMBIONT. Dale S. Mulholland* and Frank E. Friedl, Department of Biology, University of South Florida, Tampa, FL 33620.

Previous work has shown the occurrence of a hydrozoan, probably genus *Eutima*, lightly attached to gills and mantle of the eastern oyster as an inquiline symbiont (Mulholland and Friedl, J. Shellfish. Res., vol. 15). Recently, this hydrozoan was also discovered in mussels (*Geukensia demissa*) on a heavily infested oyster bar on Florida's east coast. In addition, the symbiont has been

cultured in the laboratory for up to 6 weeks. Thus, the relationship appears to be facultative with respect to the oyster host.

Adult oysters are largely herbivorous, but their filter-feeding currents also bring into the mantle cavity many small animals. Since cnidarians are generally considered to be carnivorous, the symbiont could feed without competing with its host. The finding of many hundreds of hydroid polyps among the gills of healthy-appearing, even "fat" and reproductive, oysters lends strength to this suggestion. Feeding experiments, lasting 3 weeks each, also support this hypothesis. Growth and mortality under single-food diets of marine protozoans, marine rotifers (*Brachionus* sp.), brine shrimp (*Artemia salina*) or motile marine algae (*Stephanoptera* sp.), were compared to those in starved animals. While diets of protozoans and rotifers delayed an inevitable loss of tissue mass, a brine-shrimp diet led to rapid growth of polyps and development of full-sized, free-swimming medusae within 2–3 weeks. These results are suggestive of a commensalistic oyster-hydrozoan relationship.

EVALUATION OF OYSTER SURVIVAL AND CONDITION, *VIBRIO VULNIFICUS* AND OTHER INDICATOR MICROORGANISM LEVELS IN OFFSHORE RELAYED OYSTERS. Clifford R. Vines,* Yolanda J. Brady, and Huseyin Kucuktas, Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL 36849; Angelo DePaola and Miles Motes, U.S. Food and Drug Administration, Gulf Coast Seafood Laboratory, Dauphin Island, AL 36528.

Oysters, *Crassostrea virginica*, were relayed from four acclimation sites with salinity and temperature ranges of 4.3 to 28 ppt and 26.5 to 38°C, respectively, to an offshore relay site with salinity and temperature ranges of 33 to 37.6 ppt and 24 to 30°C at the suspension depth of 6.6 m, respectively. Survival and condition of the oysters were evaluated at two or three week intervals at the acclimation sites and weekly at the relay site over a two to three week period during experimental trials. Condition index and liquor salinity measurements were made on individual oysters. Counts for total vibrios, heterotrophs, and fecal coliforms were determined on composites from 10 to 12 oysters. Total *Vibrio vulnificus*, heterotrophic aerobic, fecal coliforms counts and total *Vibrio* counts were determined for each sample. The level of the protozoan parasite, *Perkinsus marinus*, was determined in individual oysters. Water samples were collected weekly at the relay site from the suspension depth and *V. vulnificus* was detected, at a low level, in only one of the samples. A sharp increase in survival was noted in oysters relayed from a salinity of 9 ppt and higher as opposed to those relayed from 7 ppt and lower. *V. vulnificus* were reduced from greater than 1000 per gram to less than 10 per gram MPN; and fecal coliforms from greater than 100 per gram to less than 1 per gram MPN in oysters relayed from 9 ppt and higher salinities after two weeks at the relay site. Analysis is now in progress to

determine the effect of relaying on *Perkinsus marinus* levels and condition index.

BIVALVE CULTURE

AN APPROACH TO AQUACULTURAL PRODUCTION OF THE PENSHELL *ATRINA MAURA* SOWERBY, 1835 (BIVALVIA: PINNIDAE) IN NORTHWEST MEXICO. Francisco Cardoza-Velasco* and Alfonso N. Maeda-Martínez, Centro de Investigaciones Biológicas del Noroeste, P.O. Box 128, La Paz, B. C. S., 23000, México.

Penshell fishery has been a very important economic activity in Mexico for many years. Production trends, however, have drastically declined over the past five years and aquaculture, an alternative mode of production, is still in a developmental stage. An experimental culture of the penshell *Atrina maura*, commonly known in Mexico as "hacha" (hatchet), has been carried out in a scallop culture lease south of Bahia Magdalena, Baja California Sur to provide information on survival and growth rates as a means of evaluating penshell culture feasibility. The overall process has gone through two stages: a nine-month (March–November 1995) suspended culture in Nestier® trays in which the growth rate was 10 mm · month⁻¹ and an eleven month (December 1995–September 1996) bottom culture in which the growth rate has been 12 mm · month⁻¹. The survival rate for the first stage was of 65% while for the second stage has not been quantified yet. At the end of this period the mean wet weight for the adductor muscle, which is the eatable portion of this species was of ca. 14 g, with no significant difference between two culture densities (15 and 75 individuals/m²). Although the expected harvest time for *Atrina maura* is two years, which is longer than that needed for other bivalves such as oysters and scallops, its high price in Mexico's domestic market (16.80 U.S. Dlls/Kg) makes it a very attractive species.

OPTIMIZING TRIPLOID PRODUCTION TECHNIQUES AND COMPARATIVE FIELD PERFORMANCE OF MEDITERRANEAN MUSSELS (*MYTILUS GALLOPROVINCIALIS*) IN PUGET SOUND. Jonathan P. Davis, Taylor Resources, Quilcene, WA 98376.

Triploid mussels (*Mytilus galloprovincialis*) were produced using systematic combinations of heat shock and the purine 6-dimethylaminopurine in order to determine the optimal treatment program for producing commercial quantities of triploids. Fertilized eggs treated to a five or ten degree C temperature shock in combination with exposure to 6-dimethylaminopurine (30–300 µM) during the period corresponding to the release of the second polar body in the egg resulted in nearly 100% triploid larvae and

juveniles. These methods were subsequently optimized in order to routinely treat 100–300 million eggs for commercial scale triploid mussel production. Survivorship of eggs to the straight hinge stage was typically about 25%.

Survivorship and the rate of growth of juvenile mussels was subsequently followed at two commercial aquaculture facilities in Puget Sound, Washington State. Under high productivity conditions (Totten Inlet), both diploid and triploid mussels grew rapidly and survived equally well; after fourteen months mean diploid shell height was 79.8 mm while triploid mussels were slightly larger at 80.2 mm mean shell height. This difference was not statistically significant. At a second, lower productivity site, overall growth was significantly reduced in both diploid and triploid mussels, although again the difference in mean shell height between diploids and triploids after fourteen months was not significantly different. These results suggest that with respect to growth and survivorship, there are no advantages to triploidy in Mediterranean mussels.

Variation between diploid and triploid mussels with respect to gametogenic activity was also evaluated. Preliminary results suggest that triploid mussels in both high and intermediate productivity environments do undergo gametogenesis, however the extent of gonadal development and gamete maturation appears less than that of diploids. These results will be discussed in light of the potential importance of triploidy in the commercial production of cultured mussels in the Pacific Northwest.

AQUACULTURE DEVELOPMENT PARK: INDUSTRY, TRAINING AND SUPPORT PROGRAMS. Megan Davis-Hodgkins,* Mike Ednoff, and David E. Vaughan, Harbor Branch Oceanographic Institution, Inc., 5600 US 1 North, Ft. Pierce, FL 34946.

In 1995, Harbor Branch Oceanographic Institution, Inc. (HBOI) established a 40-acre Aquaculture Development Park to provide a centralized area where industry, researchers, government, and educators can collaborate on improving existing aquaculture technology, transferring new technology, and developing culture techniques. The Park supplies the user with a high quality, pre-permitted site for culturing fresh and salt warm-water species. Private industry companies operating in the HBOI Park include an upland clam farm, a shrimp hatchery, and a marine ornamental center. A hands-on educational facility, Aquaculture Center for Training, Education and Demonstration (A.C.T.E.D.) provides applied training in molluscan, crustacean and finfish aquaculture. Courses are designed to train the participants with practical experiences necessary for aquaculture employment, expanding an aquaculture operation, or implementing an aquaculture business. The Park also provides support services to the nation's aquaculture industry. A state-of-the-art clam hatchery produces seed clams for graduates of HBOI's training programs. In the future other hatch-

eries will be located in the Park to assist in industry development. The Aquaculture Development Park at HBOI is proving to be an innovative center for development and expansion of aquaculture for industry, training and support programs.

A REVIEW OF STUDIES ON THE IMPACT OF OYSTER AQUACULTURE TO WEST COAST BENTHIC INVERTEBRATE COMMUNITIES. Brett R. Dumbauld,* Washington State Department of Fish and Wildlife, P.O. Box 190, Ocean Park, WA 98640.

A review of several field studies on the influence of aquaculture practices on the benthic macro-invertebrate community in west coast estuaries suggests that the addition and removal of oysters as structural habitat plays a more important role than disturbance due to dredging and even chemical application to remove burrowing shrimp as pests. Species abundance, biomass, and diversity are often enhanced in areas where oysters are cultured versus the open mud or eelgrass dominated habitat that is replaced. Shifts in the dominant species are usually due to the presence of the oysters themselves which add structure for macro-algal attachment as well as mussels and barnacles which in turn provide protection and/or food for juvenile Dungeness crab, shore crabs *Hemigrapsus*, tube building gammarid amphipods such as *Amphithoe* and *Corophium*, caprellid amphipods, tanaids, and some annelids such as the scaleworm *Harmothoe*. Other species including the burrowing amphipod *Eohaustorius* and the commensal clam *Cryptomya*, which are adapted to live in an open sandy habitat dominated by thalassinid shrimp, are less abundant in oyster culture areas. A slightly different case is presented for off bottom culture where the structure is less likely to directly influence the benthic community, but may influence the abundance of epibenthic predators and have other structural effects. For the estuarine manager, the functional result of these species shifts and the temporal and spatial scale of disturbance are important considerations and to date little has been done to estimate functional effects at the larger estuarine ecosystem scale.

THE POTENTIAL FOR ARK SHELL CULTURE IN VIRGINIA: A COMPARISON OF TWO SPECIES. Katherine A. McGraw,* Biology Department, Radford University, Radford VA 24141; Michael Castagna, College of William and Mary, Virginia Institute of Marine Science, Eastern Shore Laboratory, Wachapreague, VA 23480.

Arkshell clams are harvested and/or cultured for consumption throughout much of the world. Although a growing fishery for arkshells, or blood clams, has developed on the Eastern Shore of Virginia over the last several years, inconsistent and dwindling supplies have hindered efforts by some local seafood processors to explore more lucrative markets. Several dealers have inquired

about the possibility of culturing *Noctia ponderosa* and *Anadara ovalis*, the two species being harvested. We have gathered life history data on these two species and compare the attributes of both as candidate species for aquaculture. Some of the factors discussed include habitat preferences, growth and survival rates, recruitment, predators and predation rates, and potential markets. *Noctia ponderosa* has a relatively slow growth rate and is more abundant, however *Anadara ovalis* has a growth rate comparable to oysters.

MANAGEMENT STRATEGIES FOR FOULING CONTROL IN ALABAMA OYSTER CULTURE. F. Scott Rikard* and Richard K. Wallace, Auburn University Marine Extension and Research Center, Mobile, AL 36615; Christopher L. Nelson, Bon Secour Fisheries, Inc., Bon Secour, AL 36511.

Fouling by marine organisms is a major impediment to the development of inshore mariculture. Fouling control methods for off bottom oyster culture were analyzed experimentally over a three year period for effects on fouling, oyster growth, oyster condition and oyster survival. Oysters were held in plastic mesh bags attached to a belt system suspended in the water column. The first year study focused on pressure washing treatments at 2, 4, and 8 week intervals, and biological control treatments using blue crabs, hermit crabs and stone crabs, and a control receiving no washing or animals. Frequently washed oysters (2 and 4 week intervals) had significantly less fouling than the 8 week wash interval or the unwashed control but were significantly smaller and suffered greater mortality. Stone crabs showed the most potential for biological fouling control but also appeared to prey significantly on the oysters. Second year treatments were a 6 week wash interval, a bag change treatment, biological treatments using larger blue crabs, and a control. There were no significant differences in fouling, mortality and condition among all treatments at the time of harvest. Some significant differences in the fouling index between 6 week wash interval and other treatments were seen during peak fouling times. There was a significant difference in growth between the bag change treatment and the control at the time of harvest. In the third year of the study, two experimental belts were set up consisting of four treatments each. Treatments included pressure washing, a saturated salt solution dip, a hydrated lime solution dip, and a control. One belt was treated only during peak settlement of fouling organisms based on a monitoring program. The other was treated at a 6 week interval. The hydrated lime solution treatment resulted in significant mortalities on both belts. Pressure washing during peak settlement significantly reduced fouling over pressure washing at a 6 week interval. Current management suggestions are to pressure wash bags at a 6 week or greater interval and also target washing to coincide with peak settlement times.

FLORIDA HARD CLAM AQUACULTURE PRODUCTION: AN EMERGING INDUSTRY. Leslie N. Sturmer,* University of Florida, Cooperative Extension Service, Cedar Key, FL 32625; David E. Vaughan, Harbor Branch Oceanographic Institution, Inc., Aquaculture Division, Ft. Pierce, FL 34946.

Retraining of former Florida fishermen in shellfish aquaculture employment opportunities was promoted by state sponsored programs. The success of the JTPA-funded Project OCEAN demonstrated this potential in 1993 and enabled displaced netfishers to be instructed in clam culture through Project WAVE during 1996 and two ongoing community-based programs. Program graduates enter into small, independent businesses by each acquiring a 2–4 acre aquaculture lease with an annual profit potential of \$30–35,000. Currently, about 200 new shellfish growers farm over 700 acres of state-owned submerged lands off Florida's west coast. Production of hard clams, *Mercenaria mercenaria*, has fast become established with statewide reports rising 400% from 8.8 million clams harvested in 1991 to over 43 million in 1995 with a respective crop value of \$5.4 million. Recent efforts have moved from focusing on production to developing infrastructure to support this emerging industry. Local manufacturers of clam bags and other equipment suppliers have become established. Emphasis has been placed on seed production with several private-sector hatcheries and land-based nurseries being developed. Another focus is marketing and distribution of Florida farm-raised clams. Technical research on shelf life and handling protocols is being evaluated to enhance harvest and storage methods. Shellfish aquaculture has revitalized fishery dependent communities, and transition from training to a sustainable industry is ongoing.

PERFORMANCE OF TRIPLOID OYSTERS IN LOUISIANA. John E. Supan,* Office of Sea Grant Development, and Charles A. Wilson, Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803; Standish K. Allen, Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

Triploid oysters (*Crassostrea virginica* [Gmelin]) were evaluated as a potential summer-crop for increasing shucked meat yield. Triploid and diploid sibling broods were reared identically until planting size (≥ 20 mm) in June–July, 1994 and distributed on bottom on separate halves of a one-acre reef plot. Mean shell height, wet meat weight, ploidy and survival were measured bimonthly for two years. Percent triploidy decreased from 85% to 56%; post-planting spatfall became indistinguishable from the triploid brood. Mean triploid shell height and wet meat weight were significantly greater ($P \leq 0.05$) than mean diploid values during November, 1994, 95 and July, 1996. Final meat yield was determined during July, 1996 by weighing individual meats from two sacks (i.e., 270 oysters) harvested from the triploid half-acre and 1 sack from diploid half-acre. Triploid oysters, verified by flow cytometry, had a 0.45 kg meat yield increase per sack over diploids.

BIVALVE PHYSIOLOGY

THE USE OF 5-BROMO-2-DEOXYURIDINE AS A PROBE TO DEMONSTRATE THAT BASIPHIL CELLS OF THE DIGESTIVE GLAND OF THE HARD CLAM, *MERCENARIA MERCENARIA*, ARE GENERATIVE CELLS. Albert F. Eble* and Victoria McCloy, Department of Biology, Trenton State College, Trenton, NJ 08650–4700.

The digestive gland in *Mercenaria mercenaria* and most bivalve molluscs, consists of two types of cells: secretory-absorptive or digestive cells and basiphil cells. 5-Bromodeoxyuridine, an analogue of thymidine, is a useful probe for the S phase of the cell cycle. After a 10-day acclimation period, clams (35–40 mm shell length) were injected with a concentrated aqueous solution of 5-bromodeoxyuridine at a dose of 1 mL/100 g wet weight soft tissue into the anterior adductor muscle. At intervals of 6, 24, 36 and 48 hours, digestive glands were excised, fixed in Davidson's solution and subsequently embedded in Paraplast and sectioned at 5 μ m. Sections were treated with biotinylated anti-5-bromodeoxyuridine and color developed with streptavidin-horse radish peroxidase and DAB; cells were counterstained with hematoxylin. Approximately 13% basiphil cells showed the positive brown color in their nuclei; secretory-absorptive cell nuclei were negative. These results prove that basiphil cells are, indeed, generative cells in *M. mercenaria*.

OBSERVATIONS ON INDUCTION OF LUMINOL-DEPENDENT CHEMILUMINESCENCE OF EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) HEMOCYTES. Frank E. Friedl,* Department of Biology, University of South Florida, Tampa, FL 33620–5150.

Chemiluminescence is a useful and exquisitely sensitive tool for biochemical and metabolic analyses. Although light production has been demonstrated with hemocytes of a number of molluscs, mechanisms of activation and signaling are not frequently addressed. Additionally, insight into sources of endogenous background chemiluminescence as well as those resulting from cell stimulation is of great importance.

A photon-counting luminometer is employed to sequentially and continuously measure light from cell, luminol, stimulant, and other experimental additions. Hemolymph volumes of 0.5 ml have been used raw or diluted and from single or pooled oysters. Typically, initial luminol-dependent light production is quite variable at a level that rises on zymosan stimulation, peaks, and decreases over several hours. Oxygen availability and/or mechanical stimulation may affect this pattern.

Since particulates appear to favor chemiluminescence, and soluble stimulants may not, phagocytotic activities may be more significant stimulating mechanisms than surface membrane reactivities. Preliminary experiments indicate EDTA, Deferoxamine, and Trifluoperazine rapidly reduce zymosan-stimulated light pro-

duction, and thus metallic ion signaling or reactivity may also be involved. Also, initial light production upon luminol addition may reflect oxidative product buildup and current endogenous oxidase activity which intensifies as cellular activity increases with stimulation.

IN VITRO MITOSES OF CLAM CARDIAC CELLS. Stephen J. Kleinschuster* and Jason Parent, Rutgers University, Haskin Shellfish Research Laboratory, Port Norris, NJ 08349; Charles W. Walker, Department of Zoology, Rudman Hall, University of New Hampshire, Durham, NH 03824; C. Austin Farley, US DOC, NOAA, National Marine Fisheries Service, Oxford, MD 21654–9724.

Up until the present time, researchers have had little success in long term, anchorage-dependent *in vitro* propagation of cells from marine mollusks. Although short term anchorage-dependent primary cultures can be relatively easily established, documented mitotic activity has been minimal. Further, long term *in vitro* replication following passage has not been demonstrated. Consequently, studies requiring such *in vitro* requisites have been impeded.

We were able to successfully culture and demonstrate sustained mitoses in cultures established from cardiac tissue of *Mya arenaria*, using medium consisting of sterile glass distilled-deionized water, 1000 ml, MEM Eagle with Earle's salts, L-glutamine, and nonessential amino acids, 4.85 g; $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$, 1.82 g; KCl, 0.68 g; $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$, 4.36 g; NaCl, 24.26 g; $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 3.16 g; HEPES buffer, 5.0 g; glucose; 0.5 g, FBS and sterile cell-free *Mya arenaria* hemolymph, each 10% by volume; and insulin/transferrin/sodium selenite supplement, 2% by volume. The cultures exhibited sustained replication in primary culture and were successfully subcultured after 6 weeks following which the cells resumed anchorage-dependence and mitoses. Hearts were dissected from clams with both normal hemocytes and neoplastic hemocytes. No appreciable difference was noted in the cultured cardiac cells from both types of clams with regard to mitotic index, nuclear/cytoplasmic ratios, chromosome morphology, or contact inhibition. A decrease of the mitotic coefficient and increased senescence was observed as the cultures aged. Based on these observations, it is expected that the generation number of the cell cultures described herein will be finite.

A HISTOLOGICAL STUDY OF DIGESTIVE TUBULES IN INTERTIDAL AND SUBTIDAL OYSTERS, *CRASSOSTREA VIRGINICA*, COLLECTED AT HIGH AND LOW TIDES. James T. Winstead, U.S. Environmental Protection Agency, Gulf Breeze, FL 32561.

Digestive diverticula from intertidal and subtidal oysters, *Crassostrea virginica*, were histologically examined to gain a better understanding of their normal morphology during high and low tides. Intertidal and subtidal oysters (total of 216) from Bayou Texar, Pensacola, Florida, were collected adjacent to each other at

either high or low tides or during two complete tidal cycles. Animals were shucked immediately and a one cm section was processed for histological examination. A digestive tubule ratio for each oyster was determined by measuring an inside to outside tubule value from 20 tubules per animal. Digestive tubules with high tubule ratios had squamous epithelia, while tubules with low ratios had columnar epithelia. Intertidal oysters sampled 13–15 hrs after emersion at low tides had no crystalline styles and average tubule ratios of .477 (.053SE) to .674 (.022SE) while subtidal oysters sampled had crystalline styles and average tubule ratios of .063 (.003SE) to .139 (.057SE). Intertidal oysters sampled 6–14 hrs after submersion at high tides had crystalline styles present with tubule ratios of .162 (.062SE) to .071 (.001SE) and subtidal oysters also possessed crystalline styles with tubule ratios between .076 (.004SE) and .098 (.004SE). These data indicate that intertidal *C. virginica* respond to tidal cycles by losing or reconstituting the crystalline style concomitant with changes in tubule morphology. In contrast, digestive tubules in subtidal oysters were not affected by normal tidal cycles, supporting the contention that they are continuous feeders.

CONTEMPORARY ISSUES OF THE GULF OF MEXICO OYSTER INDUSTRY

MEETING THE GULF OF MEXICO PROGRAM'S SHELLFISH CHALLENGE: A PROGRESS REPORT ON THE BARATARIA/TERREBONNE BAYS IMPLEMENTATION ASSESSMENT PROJECT. Daniel R. G. Farrow, C. John Klein, Anthony S. Pait, and Brian Johnson, NOAA, Strategic Environmental Assessments Division, 1305 East West Highway, Silver Spring, MD 20910; Frederick Kopfler and Thomas Herington, Gulf of Mexico Program, Building 1103, Room 202, Stennis Space Center, MS 39529–6000; Brent Ache, Battelle, 365 Canal Street, Suite 2300, New Orleans, LA 70130.

In February 1994, members of the Gulf of Mexico Program and the Strategic Environmental Assessments Division of the National Oceanic and Atmospheric Administration (NOAA) began a project to make progress on the Shellfish Challenge, one of 10 Environmental Challenges developed by the Program to address coastal environmental problems in the Region. The first or strategic assessment phase of the Shellfish Challenge Project involved over 85 regional specialists in shellfish management and pollution control, and resulted in the development of 32 strategies and the identification of 24 watersheds in the Gulf of Mexico where the strategies would have the greatest chance of being successfully implemented. The second phase of the project will involve a series of implementation assessments to explore the feasibility of successfully undertaking priority restoration activities in selected watersheds. These assessments will capture information on the time frame, cost, financing, institutions involved, regulations, impacts on stakeholders, indirect impacts, and the role of the Gulf of Mexico

Program that will be used by state and local stakeholders to decide which projects are most feasible to implement. The first implementation assessment is being conducted in the Barataria/Terrebonne Bays estuarine system. Representatives from federal, state and parish governments along with the shellfish industry and academia are being asked to participate to ensure a broad representation of views and a consensus on the most appropriate and realistic strategies to implement in the Barataria/Terrebonne system. The implementation assessments will provide the detailed, watershed-level characterization needed before actual strategy implementation can proceed. They will not only lay the foundation for strategy implementation in the targeted watershed, but will also provide insight into the potential transferability of implementation techniques and strategies to other watersheds in the region.

THE OYSTER INDUSTRY IN FLORIDA. John Gunter,* James Marshall, and Mark Berrigan, Florida Department of Environmental Protection, Tallahassee, FL 32399.

Florida, like other Gulf Coast States, has experienced significant variations in oyster production over the past ten years. These variations have resulted from a combination of factors, including environmental conditions, stock abundance, and market trends. Instability associated with harvest levels and dockside values have resulted in substantial changes in the oyster fishery. The oyster industry in Florida remains concentrated in Apalachicola Bay, but other estuaries that historically supported commercial harvesting are now only marginal or non-commercial producers. Oyster resource assessments in productive growing areas confirm variations in stock abundance, but stock abundance does not account for all of the changes in the fishery. In marginal producing areas, oysters have provided an opportunistic harvest for fishermen that were not dependent on oysters as their sole fishery product. Recently, cumulative impacts on other fisheries are also affecting the oyster fishery, as fishermen move away from fishing altogether. This paper provides an overview of Florida's oyster fishery and describes current programs to develop oyster resources.

ENVIRONMENTAL ISSUES FACING LOUISIANA'S OYSTER INDUSTRY IN THE 1990S. Earl J. Melancon* and Thomas M. Soniat, Department of Biological Sciences, Nicholls State University, Thibodaux, LA 70310; Ronald J. Dugas, Louisiana Department of Wildlife and Fisheries, 1600 Canal St., New Orleans, LA 70112.

Louisiana has 25% of the nation's coastal wetlands, but is also experiencing 80% of the nation's annual loss. Mississippi River water diversions are being constructed to slow the processes of wetlands loss. These projects are changing seasonal and yearly salinity patterns within the estuaries. This has created fresher conditions on some oyster leases while stimulating oyster production in other areas. The Louisiana oyster industry was among the first proponents of freshwater diversion into the estuaries, but there is a need to find a reasonable solution to the negative impact to the

leases close to each outfall. An Oyster Mitigation Task Force has been established to seek a solution to this stalemate, including the potential for monetary compensation or relocation. Louisiana is also developing a method to evaluate the potential productivity of an oyster lease and has begun efforts to develop a valuation matrix. The valuation matrix should be useful in resolving the diversion issue and will also be beneficial in addressing the negative impacts on leases due to oil and gas exploration.

Another significant issue is sewage pollution, especially from non-point sources. *Vibrio vulnificus* is a high priority issue and the oyster industry is adjusting to the Food and Drug Administration's harvest water time/temperature matrix designed for the control of *V. vulnificus* by quick refrigeration. The Barataria-Terrebonne estuaries have been chosen as the first Gulf site for EPA's Shellfish Challenge Program to increase shellfish harvest waters by 10%.

CONTEMPORARY CHALLENGES AND PROSPECTS FACING THE OYSTER INDUSTRY IN MISSISSIPPI. William S. Perret,* Michael Buchanan, Michael Brainard, and Christine Johnson, Mississippi Department of Marine Resources, 152 Gateway Drive, Biloxi, MS 39531.

Mississippi oysters have long provided a way of life as well as recreation to many of its coastal inhabitants. It has been reported that oysters have been harvested commercially since as early as 1699.

Landings data illustrates annual production and how it has fluctuated greatly over the years. The authors discuss these trends and offer possible explanations for these annual fluctuations. Mississippi's oyster growing waters have regulatory limitations on legal harvest due to a variety of reasons. These range from administrative rules to waters not meeting criteria for water quality standards for harvest of shellfish.

Contemporary issues facing the Mississippi oyster industry are variable; they include six general categories. These are: (1) Biological, (2) Marketing, (3) Labor Force, (4) Enforcement, (5) Socioeconomic, and (6) Political. The authors discuss each of these categories and provide insight to them.

Increased oyster production is needed and can be achieved by a combination of natural and man made factors. This increased harvest would increase the economic worth and strengthen the diversity of the economic (fishery) base and provide help for displaced commercial fishermen from other fisheries.

PRESENT AND FUTURE CHALLENGES FACING THE TEXAS OYSTER FISHERY. Sammy Ray, Texas A&M University, P.O. Box 1675, Galveston, TX 77553; Richard L. Benfield,* Texas Parks & Wildlife Department, Seabrook, TX 77586.

Texas ex-vessel oyster production ranged from 888,800 pounds of meat (ex-vessel value, \$1,048,700) in 1979 to 7,940,700 pounds (\$11,336,700) in 1983. The 1995 season yielded 4,670,062 pounds (\$8,792,024). Texas oysters experience multiple factors that may

impact landings. The most serious threat to oysters in Texas bays is reduced freshwater inflow. Texas oysters thrive in salinities ranging from 10–20 ppt. Increased mortalities and diseases can occur in salinities ranging from 20–25 ppt or greater. High salinities are conducive to oyster drill (*Thais haemastoma*) and dermo (*Perkinsus marinus*) incidence, both can decimate oyster populations. Bottom substrates such as oystershell or clamshell are important factors in oyster propagation.

Domestic and industrial pollution, saltwater intrusion (ship channels), fishing exploitation, and seismic exploration are also factors to be considered. Adverse publicity due to illnesses caused by *Vibrio vulnificus* can impact marketing of Texas oysters. Availability of oysters in Gulf and Atlantic states impacts demand for Texas oysters. Heavy spat setting was observed on reefs in Galveston, Matagorda, and San Antonio Bays during 1996. Survival to market size depends upon the aforementioned factors. The Texas Parks & Wildlife Department monitors oyster populations monthly with oyster dredges. Commercial oyster dealers report monthly landings of oysters. Data collected are used to evaluate of environmental and other factors upon oyster production.

CURRENT AND FUTURE PROSPECTS FOR THE OYSTER INDUSTRY OF ALABAMA. Mark S. Van Hoose,* Alabama Marine Resources Laboratory, P.O. Box 189, Dauphin Island, AL 36528.

The productivity of Alabama oyster reefs at present is not threatened by coastal development but remains limited by lack of cultch material. Funding of reef rehabilitation projects is a chronic problem but in the future, even given sufficient monies, there is likely to be less shell available. Alabama has traditionally processed four to five times the amount of oysters it has harvested. This imported shell supply will diminish when other Gulf states enact measures to preserve their native shellstock. A new, economically feasible, alternative cultch material needs to be identified if Alabama reefs are to be maintained at their current levels of harvest.

An ominous sign of change in basic Gulf of Mexico dynamics, as indicated by Alabama's first red tide, foreshadows a new threat to the oyster industry. Work to determine if this recent event is an anomaly or signals a major new development is needed.

CRAB FISHERIES

CONTINUING DECLINE IN SIZE OF MALE BLUE CRABS IN MARYLAND. George R. Abbe,* Estuarine Research Center, Academy of Natural Sciences, 10545 Mackall Road, St. Leonard, MD 20685.

With an apparent decrease in blue crab landings in the Chesapeake Bay during the past 5 or 6 years, we have continued to examine data from crab catches near Calvert Cliffs, Maryland collected from 1968 through 1996 in an effort to gain some un-

derstanding of a cause. As we reported in 1996, the annual mean size and weight of female crabs have remained relatively stable, but the mean size and weight of males have decreased significantly. By separating sublegal-size crabs (<5 in carapace width) from legal-size, and sorting legal-size into 1-in size classes, we have gained further insight into the decreasing size of male crabs.

Sublegal females and the three legal female classes (5–6, 6–7 and >7 in) showed no significant trends when examined by linear regression. Males, however, showed significant trends for all size classes. Sublegal males increased from 24% of the male population during the first 5 years of the study (1968–72) to 37% during 1980–84 to 65% during the last 5 years. All classes of legal males, however, exhibited downward trends. Males 5–6 in decreased from 45% of the male population in the earliest period to 41% in the middle period to 29% during the last 5 years. Males 6–7 in decreased from 27% during 1968–72 to 20% during 1980–84 to only 6% during 1992–96. Males 7 in and larger accounted for 4% of the males in the earliest period, but decreased to 2% in the middle period, and were down to 0.4% for the most recent time. These size decreases for the most valuable portion of the blue crab population are further evidence of over exploitation. These trends might be reversed by additional regulations aimed at reducing effort, but an increase in minimum legal size of $\frac{1}{4}$ to $\frac{1}{2}$ in would possibly be more effective. Such an increase would allow many male crabs an additional molt which would put them into a size class larger than 6 in and lead to an increase in mean size of males and an increase in landings without the addition of more crabs. An increase in minimum legal size for females would probably not result in many molting to larger size, but it would protect significant numbers of additional spawners.

LONG TERM TRENDS IN BLUE CRAB ABUNDANCE IN LOUISIANA. Vincent Guillory and Paul Prejean, Louisiana Department of Wildlife and Fisheries, P.O. Box 189, Bourg, LA 70343.

Long term trends in abundance of blue crab (*Callinectes sapidus*) was obtained from the inshore fishery independent bottom-fish/shrimp assessment and monitoring program of the Louisiana Department of Wildlife and Fisheries. Samples were taken with a 16-foot flat otter trawl from approximately 25 inshore stations weekly from March to October and biweekly from November to February. Blue crabs were counted, sexed, and carapace width (CW) of 50 individuals measured in 5 mm intervals. Annual catch per effort (CPE) of early juvenile blue crabs <40 mm CW and of CPE of adult (≥ 125 mm CW) blue crabs fluctuated from year to year, although there was a significant upward trend in the former and a significant downward trend in the latter. These data suggests that blue crab populations in Louisiana are probably limited by postsettlement processes. Associated with the divergent trends in CPE of different sizes of crabs was a long term decrease in mean

size of blue crabs. There was no consistent relationship between early juvenile CPE and later adult CPE. A complex of interacting, nonquantifiable factors probably contributed to long term trends in early juvenile and legal blue crab CPUE.

STOCK ASSESSMENT OF BLUE CRABS IN THE GULF OF MEXICO: PERSPECTIVE AND PROBLEMS. Harriet M. Perry, Vince Guillory,* Tom Wagner, Philip Steele, and Stevens Heath, Blue Crab Technical Task Force, Gulf States Marine Fisheries Commission, P.O. Box 726, Ocean Springs, MS 39564.

Assessment of blue crab stocks in the Gulf of Mexico is hampered by the lack of reliable fishery dependent data. Additionally, the multi-state nature of the fishery with varying statistical and sampling methodologies, and the existence of geographically separated, ecologically distinct management units further complicates estimates of population size. Although current genetic evidence suggests that blue crab populations in the Gulf of Mexico are homogeneous, wide exchange between geographic areas may be limited by physical barriers to dispersal (i.e., the Mississippi River). Based on current knowledge of migratory patterns, stock units may include, but not be limited to: peninsular Florida, the Florida panhandle, the north central Gulf of Mexico east of the Mississippi River, Louisiana west of the Mississippi River, and Texas. With the exception of the north central Gulf, fishery independent sampling methodologies for postlarvae and juveniles are not standardized. Use of these fishery independent data sets from individual states to address stocks that range over wide geographic areas further hampers assessment of population size. Finally, the apparent lack of relationships (spawner/recruit, settlement/recruitment, etc.) which typically characterize conventional stock assessment models forces assumptions not supported by data.

FISHERY INDEPENDENT SAMPLING FOR MEGALOPAE AND JUVENILE BLUE CRABS IN MISSISSIPPI COASTAL WATERS. Harriet M. Perry,* James Warren, and Christine Trigg, Gulf Coast Research Laboratory, P.O. Box 7000, Ocean Springs, MS 39564.

A variety of sampling methodologies are used to determine relative distribution and abundance of megalopal and juvenile blue crabs in Mississippi waters. Juvenile blue crabs are sampled monthly with otter trawls, bag seines, and beam plankton nets at selected stations in the Biloxi Bay estuarine system. Data on juvenile abundance are available from 1974 through the present. Monitoring of megalopal settlement began in 1991 and continues to date. Settlement is monitored daily from May through October using four stationary surface collectors suspended from a pier. Annual variations are evident in recruitment of small crabs to the juvenile population and in the total number of crabs collected.

Although statistical analyses show downward trends in overall juvenile catch, catch by gear type and catch by 10.0 mm size class, coefficients of determination are low and little of the variability is accounted for by the mathematical models. Numbers of megalopae on collectors declined from 1991 through 1996. Preliminary analysis of meteorological conditions associated with extremely low megalopal settlement in 1996 suggests that wind conditions and shelf circulation features created unfavorable hydrographic conditions for movement of larvae and postlarvae into Mississippi coastal waters.

FISHERY-INDEPENDENT MONITORING OF THE BLUE CRAB (*CALLINECTES SAPIDUS*) IN TEXAS COASTAL WATERS. Tom Wagner,* Texas Parks and Wildlife Department, Rockport, TX 78382.

Routine fishery-independent monitoring programs are used by the Texas Parks and Wildlife Department to determine long-term trends in relative abundance and distribution of blue crabs (*Callinectes sapidus*). Coastwide gill nets since 1975, bag seines since 1977, bay trawls since 1982 and gulf trawls since 1985 were used to determine relative trends in catch rates and mean size of blue crabs. Catch rates of larger crabs are declining, while catches of smaller crabs are level or increasing, indicating stable recruitment. Long-term declines in mean size of crabs are evident. These data are used in conjunction with fishery-dependent data and occasional special studies to provide a sound biological basis for blue crab fishery management in Texas coastal waters.

BEHAVIORAL RESPONSES OF RED KING CRAB TO CRAB POTS AND THE APPLICATION IN POT DESIGN. Shijie Zhou,* Alaska Department of Fish and Game, DCFMD, P.O. Box 25526, Juneau, AK 99802; Thomas C. Shirley, Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Juneau, AK 99801.

The red king crab fishery in Alaska had 64.6% bycatch of female and sublegal-sized male crabs during 1991–93; the efficiency of crab pots currently being used needs to be improved. This study examined behavioral responses of red king crabs to pots under laboratory conditions. Crabs approached the pot from downstream and 78.3% of crabs searched less than 90° before leaving or entering. The probability of entry increased with the number of approaches with an average success rate of 8.1%. No significant differences in approach, search, and entry were found between ovigerous females, juvenile females, legal-sized males, and sublegal-sized males. The high entrances prevented crabs from escaping. Crabs depended on chemical cues during foraging, approaching, and searching. The current king crab pot was inefficient because crabs had difficulties in accessing the entrances and non-legal crabs had difficulties in escaping. Based on these observations, a new crab pot was designed to increase the catch of

legal males while reducing the catch of female and sublegal male crabs. Under laboratory conditions the new pot design was found to be superior to the pot design in current use.

CRAYFISH

REPRODUCTION IN TWO SPECIES OF PROCAMBARID CRAWFISHES, *PROCAMBARUS CLARKII* (GIRARD, 1852) AND *PROCAMBARUS ZONANGULUS* HOBBS & HOBBS 1990, IN SIMULATED BURROWS. Jay V. Huner and T. Blair Shields, II, Crawfish Research Center, University of Southwestern Louisiana, Lafayette, LA 70504; J. Patrick Bohannon, Mark Konikoff, and David Guilmet, Department of Biology, University of Southwestern Louisiana, Lafayette, LA 70504.

Procambarus clarkii and *Procambarus zonangulus* are important commercial crawfish species that frequently co-exist in natural waters and aquacultural impoundments. Both species reproduce in simple earthen burrows 1.0–1.5 m deep. Efforts to spawn the two species in hatchery systems that simulate burrows have been disappointing. Our research has shown that the addition of soil to simulated burrows can result in a substantial increase in reproductive success of both species as measured by the number of resulting free living third stage juvenile crawfish. The systems without soil are much more acidic (pH = 4–5) than those with soil (pH = 6–7). Two production strategies have been compared—placing mature females in simulated burrows in mid-late spring before ovaries are developed or holding them in tanks and placing them in simulated burrows in mid-late summer when ovaries are well developed. The latter strategy appears to be best because mortality of *P. clarkii* in holding tanks can be excessive as an apparent result of chronic vibriosis. Other findings show that *P. clarkii* is generally twice as fecund as *P. zonangulus* and that neither species will oviposit in the absence of free water in simulated burrows even though they can survive several months in a humid atmosphere. Typical numbers of young for the two species are 200–300 for *P. clarkii* and 100–200 for *P. zonangulus*.

POLYCULTURE OF RED CLAW CRAYFISH (*CHERAX QUADRICARINATUS*) WITH NILE TILAPIA (*SEROTHERODON NILOTICUS*). Srikanth R. Kotha* and David B. Rouse, Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL 36849.

Polycultures of compatible aquatic species have been used to improve economic and/or ecological feasibility of aquaculture systems. One polyculture system that would appear to be compatible is the polyculture of red claw crayfish and a non-carnivores tilapia. An experiment to evaluate this possibility was conducted in nine earthen ponds at Auburn University during the summer of 1996. Juvenile red claw were stocked in all ponds at a density of 3/m². Tilapia were stocked into six ponds at a density of 1/m². In three

ponds the fish were confined in cages while they remained free swimming in three ponds. Forage, pellets and aeration were added at recommended rates to provide for good growth during an 18-week culture period. Final red claw size in the monoculture treatment (65 g) was significantly larger than either polyculture treatment (49 and 40 g). Survivals of red claw were also significantly higher in monoculture (69%) than in either polyculture treatment (52 and 50%). These results indicate that tilapia do not appear to be a suitable species for a red claw-tilapia polyculture, even when the tilapia are confined in cages.

RELATIVE CONTRIBUTION OF DIFFERENT FOOD SUPPLEMENTS TO GROWTH OF CRAWFISH (*PROCAMBARUS CLARKII*). W. Ray McClain, Rice Research Stn., La. Agri. Exp. Stn., LSU Agri. Ctr., Crowley, LA 70527-1429.

Crawfish production in the southern USA relies on established forage crops to fuel a detrital-based food-web system. This system often becomes inadequate for maximum production. Since feeding strategies utilizing formulated feeds have rarely been cost effective, this study was conducted to determine the relative contribution to crawfish growth of low-cost, single feedstuffs fed as supplements to the detrital system. Twelve-week feeding trials were conducted in replicated 38-L flow-through tanks supplied with soil, established vegetation, and pond water to provide micro-habitats that simulated pond culture environments. Hatchling crawfish (<0.1 g) were individually stocked and represented a density of 12 crawfish/m². Standing rice substrate (with seedhead removed) provided the forage base and was allowed to fragment naturally. Supplemental feed treatments consisted of (1) no additions, (2) rice stalks, (3) rough rice seed, (4) whole soybean, and (5) formulated 25% crude protein pellets. Feeds were fed 3 days/week at 28 kg/ha/day dry weight initially, increasing to 112 kg/ha/day. Crawfish receiving no supplementation were consistently smaller after 12 weeks and were below the minimal acceptable market size of 13 g. Only crawfish receiving rice seeds, soybeans, and pellets obtained the desired market size of 20 g or larger. Pellets and soybeans contributed to the largest crawfish. Crawfish receiving supplemental rice stalks were only slightly larger than the controls. These results indicate the potential limitation of forage as the sole resource for maximum growth. Whole soybeans appear to be the most desirable agronomic product for supplementation.

A QUEST TO DETERMINE CRAYFISH CONDITION. Hakan Turker and Arnold G. Eversole,* Department of Aquaculture, Fisheries and Wildlife, Clemson University, Clemson, SC 29634-0362.

A review of the literature indicates measures of crayfish "well-being" involve either destructive techniques such as hepatopancreas and abdominal muscle moisture content or length-weight relationships and condition factors. Some of these may be more

useful than others in determining the reproductive condition of crayfish. Lipids play an important role in the reproduction of crayfish; but unfortunately, traditional methods of determining lipid require sacrificing the organism. Recently, a rapid and nondestructive method has been used successfully to measure the total body electrical conductivity (TOBEC) to estimate lean body mass in live animals. Significant relationships between TOBEC readings and lean body mass have developed for a variety of vertebrates. Total body lipid estimates are possible using lean body mass and live weight. This presentation will outline our attempts to estimate lean body mass and total body lipid in individual live crayfish.

CRUSTACEAN HEALTH

RHIZOCEPHALAN PARASITES AND THEIR DECAPOD HOSTS. Henrik Glenner* and Jens T. Høeg, Department of Cell Biology and Anatomy, Zoological Institute, University of Copenhagen, Universitetsparken 15, DK-2100, Copenhagen, Denmark.

The Rhizocephala outrank most other metazoans parasites in their degree of specialization. This is especially apparent in their larval biology, their sexual system, and in the ability to affect and control their hosts. Many aspects of the life cycle such as the presence of separate sexes are critical for understanding rhizocephalan population dynamics and their ability for dispersal. The Rhizocephala parasitize other Crustacea, principally Decapoda, and reports from non-crustacean hosts are erroneous. Most rhizocephalans will assume complete control of their host, which is normally castrated. Male hosts are morphologically, behaviorally and physiologically feminized. Rhizocephalan host specificity is usually lax and one species parasitize more than 10 different host species. Rhizocephala occur in most marine and in many brackish environments and prevalence can approach 100%, but how this affects host population dynamics is largely unknown. Several commercially important Crustacea, e.g., *Portunus pelagicus*, *Callinectes sapidus* and several lithodid king crabs, suffer from Rhizocephala. But Rhizocephala are also under study for use in fighting introduced marine pests such as the European green crab *Carcinus maenas* which threatens fisheries and local environments several places in the world.

CHITINOCLASIA PREVALENCE ON AMERICAN LOBSTER (*HOMARUS AMERICANUS*) POPULATIONS IN OFFSHORE CANYONS LOCATED NEAR THE DEEP-WATER-DUMPSITE 106 (DWD-106). Diane Kapareiko,* John Ziskowski, Richard Robohm, Anthony Calabrese, and Jose Pereira, NOAA, NMFS, Northeast Fisheries Science Center, Milford, CT 06460; Regina Spallone, NOAA, NMFS, Northeast Regional Office, Gloucester, MA 01930.

During the period 1990–1992, 15,004 lobster from 146 commercial catches from traps deployed in nine offshore canyon sites

surrounding the 106-Mile Sewage-Sludge Disposal Site were examined for signs of shell disease. Overall, 1,184 lobster (7.9%) had lesions. Female lobster were more affected by this condition than male lobster. Shell-lesion occurrence was independent of carapace length. Disease prevalences in female lobster from canyons within the "potential area of influence" of the 106-Mile Site were significantly higher than those from canyons located outside of this area. However, logistic regression tests showed an equally strong regression of shell lesion occurrence with proximity to the old 12-Mile Dumpsite as that shown by proximity to the 106-Mile Dumpsite. Shell disease prevalences in a smaller sample of lobster examined during groundfish survey cruises were not significantly different from those in commercial catches. A cause and effect relationship between sewage-sludge dumping at the deep-water dumpsite and shell-disease occurrence could not be proven because of the possible existence of a 12-Mile Site effect and other factors. The possibility of multiple influences from both the 106-Mile Site and the 12-Mile Site on offshore shell-disease prevalence could not be discounted. Further analysis based upon a lesion severity index (currently in progress) may help to clarify possible dumpsite effects.

THE EFFECT OF HOST SIZE ON VIRULENCE OF TAURA SYNDROME VIRUS (TSV) TO THE MARINE SHRIMP *PENAEUS VANNAMEI* (CRUSTACEA: PENAEIDAE). Jeffrey M. Lotz,* University of Southern Mississippi—Institute of Marine Sciences, Gulf Coast Research Laboratory, P.O. Box 7000, Ocean Springs, MS 39566–7000.

Taura Syndrome (TS) which is caused by Taura Syndrome Virus, is the most important disease of the farmed penaeid shrimp *Penaeus vannamei* in the Western Hemisphere. One possible tactic to offset Taura Syndrome Virus-induced mortalities is for culturists to use larger shrimp for stocking ponds. The study consisted of 4 experiments designed to test the hypothesis that *P. vannamei* becomes more tolerant of TSV infections as they become larger. Experiments were done in either 100-L glass aquaria or 4000-L cylindrical fiberglass tanks. All shrimp used in experiments were Specific-Pathogen-Free *Penaeus vannamei* derived from United States Shrimp Farming Program Population 1. The TSV in all experiments originated from infected farm-reared shrimp collected during a 1995 TS outbreak in Texas, USA. Experimental shrimp were inoculated with virus either per os by allowing shrimp to feed on macerated infected shrimp tissue or intramuscularly by injection of a viral suspension into the abdominal musculature. In the four experiments 9–14 d survival ranged from 0% to nearly 60%. Analysis of each of the 4 experiments by logistic regression revealed a consistent trend for larger shrimp to be more likely to succumb to infection; however, the effect was only statistically significant in 2 of the 4 experiments. The results of the experiments failed to support the hypothesis that *P. vannamei* increases

its tolerance to TSV as it increases in size between 1 g and 30 g. Funded in part by USDA, CSREES Grant No. 96-38808-2580.

EPIZOOTIOLOGY AND DISEASE PROGRESSION OF A PARASITIC DINOFLAGELLATE INFECTING BLUE CRABS. Gretchen A. Messick, National Marine Fisheries Service—NOAA, Cooperative Oxford Laboratory, 904 S. Morris Street, Oxford, MD 21654–9724.

The parasitic dinoflagellate, *Hematodinium* sp., infects many crustacean species including the commercially valuable blue crab, *Callinectes sapidus*. Field studies conducted since 1992 investigating the prevalence and distribution of the parasite in blue crabs from coastal embayments along the Atlantic and Gulf of Mexico indicate that parasite prevalence follows a seasonal cycle. The parasite appears to be widely distributed with considerable variation in prevalence among sampled locations. Parasite prevalence and infection intensity are inversely related to crab size. Physical parameters of embayments such as salinity, temperature, depth, water mixing, tidal flow, and distance from inlets appear to influence prevalence of the parasite in blue crabs. Results from experiments assaying disease progression are paradoxical. Infection intensity increased in infected crabs held in flow-through coastal bay seawater, whereas infection intensity decreased in infected crabs held in static artificial seawater at a controlled temperature. Effects of this parasite on host mortality are unclear. With infections being heaviest and most prevalent in juvenile crabs, the parasite may be removing many blue crabs from coastal bay fisheries.

BACULOVIRUS PENAEI (BP) AND TAURA SYNDROME VIRUS (TSV) IN PENAEID SHRIMPS. Robin M. Overstreet,* Gulf Coast Research Laboratory, University of Southern Mississippi, P.O. Box 7000, Ocean Springs, MS 39566–7000.

The two viruses, *Baculovirus penaei* (BP) and Taura syndrome virus (TSV), produce mortalities of penaeid shrimps in the Western Hemisphere. The double-stranded DNA, BP baculovirus infects the nuclei of the midgut-proper and hepatopancreatic tubules, primarily producing diagnostic polyhedra in the hypertrophied nuclei and causing mortalities of larval and early postlarval individuals of select species, including *Penaeus aztecus* and *P. vannamei*. In contrast, the single-stranded RNA, TSV picornavirus infects the cytoplasm of cuticular epithelium and in some cases adjacent subcuticular connective tissue and striated muscle fibers. It causes multifocal necrosis with diagnostic "peppered" lesions in and mortality of postlarval to adult shrimps, including *P. vannamei*, *P. setiferus*, and *P. stylirostris*. Both viruses infect natural as well as cultured stocks of shrimps. Because of differences in their biological characteristics as well as characteristics of different penaeid stocks, responses by their hosts in hatcheries (BP), nurseries and ponds (TSV), and natural habitats (both) differ. Research using

bioassay and other methods to test many but not all of these features, including host growth, host susceptibility, and virulence, transmission, and control of the viral agents are now partially understood or under study. These results should permit better management of the respective cultured shrimp stocks. Funded in part by USDA, CSREES Grant No. 96–38808–2580.

PREVALENCE OF *HEMATODINIUM PEREZI* IN BLUE CRABS FROM CHESAPEAKE BAY, VIRGINIA. Jeffrey D. Shields,* Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062.

Hematodinium perezii is an unusual parasitic dinoflagellate that lives in the hemolymph of brachyuran crabs. The parasite is found along the eastern seaboard of the USA where it occurs in epizootics in the commercially important blue crab. Infections are probably fatal. Epizootics are associated with high salinities, and in some cases with poorly draining estuaries. The parasite is prevalent in the seaside bays of the Delmarva Peninsula from mid-late summer through winter, but it appears in the lower reaches of Chesapeake Bay in the fall and winter when female crabs are migrating to high salinity waters. In October, 1996, the prevalence of the disease along the Virginia portion of the Delmarva Peninsula varied from 20–50% in legal crabs. Lower prevalences (1–10%) were noted for crabs caught between Cape Henry, and Cape Charles. In November, the prevalence of the disease was notably higher in crabs caught between Cape Henry and Cape Charles (10–30%). The disease can spread into the breeding grounds of adult female crabs, but the prevalence is generally low during the prebreeding and ovigerous season. In 1996, rainfall was very high in many of the watersheds of the bay, and the Delmarva Peninsula. The lower salinities coupled with cooler average temperatures may have limited the spread of the parasite this year.

SOME DISEASES OF NORTHEASTERN PACIFIC COMMERCIAL CRABS. Albert K. Sparks* and J. Frank Morado, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Alaska Fisheries Science Center, Resource Assessment and Conservation Engineering Division, Alaska Fisheries Center, 7600 Sand Point Way Northeast, Seattle, WA 98115.

Investigation of the role of infectious diseases in the population structure of commercial crabs of the northeastern Pacific were initiated in 1978. Effort was intensified in 1982–1983 through cooperation with the Alaska Department of Fish and Game (ADF&G) and, especially, inclusion of shipboard necropsies of Tanner, snow, and king crabs into Alaska Fisheries Science Center and ADF&G annual crab population surveys. Over the years, several thousand crabs have been collected and examined. A large number of disease causing agents have been identified, but only a few are believed to cause significant mortalities in Northeast Pacific, commercially important crabs; they include: a Chlamydia-

like organism and a parasitic ciliate (*Mesanoophrys pugettensis*) of the Dungeness crab (*Cancer magister*); an invasive fungus (*Trichomaris invadens*) in the Tanner crab (*Chionoecetes bairdi*); a parasitic dinoflagellate in *C. bairdi* and the snow crab *C. opilio*; a Herpes-like virus disease in blue (*Paralithodes platypus*) and golden (*Lithodes acquispina*) king crabs; a pansporoblastic microsporidian (*Thelohania* sp.) in the red (*P. camtschatica*) and blue king crabs, and rhizocephalan infections (*Briarosaccus* sp.) in red and golden king crabs. Prevalence trends and the effects on the host of each disease causing agent will be presented.

IN VITRO CULTURE OF *HEMATODINIUM PEREZI* FROM THE BLUE CRAB, *CALLINECTES SAPIDUS*. Diana M. Whittington,* David S. Fridley, Valerie L. Harmon, and Jeffrey D. Shields, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Hematodinium perezii is an unusual parasitic dinoflagellate that lives in the hemolymph of the blue crab, *Callinectes sapidus*. The parasite occurs in frequent epizootics along the eastern seaboard of the USA. Little is known of the parasite's life cycle, except that in its host it occurs as a plasmodium, vegetative troph, and dinospore. Culture attempts have been moderately successful on related species. A partial progression of the life cycle was observed using filtered hemolymph for *Hematodinium* sp. in Tanner crabs. Various balanced salt solutions have been attempted for *Hematodinium* sp. in Tanner crabs, and *H. australis* in Australian sand crabs. Balanced salts media appear to maintain *Hematodinium* spp. for short periods but with little or no progression through different life history stages. A balanced salt media with bovine fetal serum has been used successfully with *Hematodinium* sp. from the Norway lobster. Our attempts with *H. perezii* using that medium were not successful. We report the successful culture of *H. perezii* from the American blue crab. We used a balanced salt medium with dinoflagellate-supporting trace metals, bovine fetal serum, and antibiotics. The original culture was initiated from a crab with a heavy, late stage infection (sporonts). Several different life history stages have been observed in cultures including dinospores, highly motile plasmodia, trophonts, and a potential cyst stage.

CURRENT ISSUES AND SOLUTIONS FOR SHELLFISH SANITATION PROGRAMS

ASSESSMENT OF THE FLORIDA *VIBRIO VULNIFICUS* TIME/TEMPERATURE HARVEST CONTROL MATRIX. David C. Heil* and Mark L. Collins, Florida Department of Environmental Protection, 3900 Commonwealth Boulevard, Tallahassee, FL 32399.

The National Shellfish Sanitation Program incorporated *Vibrio vulnificus* time/temperature harvest controls in 1996. These regulatory controls were established to reduce the risk associated with the naturally occurring bacteria *Vibrio vulnificus* in bivalve

molluscan shellfish. This paper presents the state of Florida's experience with implementation and compliance of these regulatory controls. Assessments are made concerning the effectiveness of implementation and compliance of these regulatory controls. Preliminary assessments are made concerning effectiveness of the time/temperature harvest control matrix on reported *Vibrio vulnificus* infections from Florida bivalve molluscan shellfish.

DISCRIMINATION OF POINT AND NON-POINT SOURCES OF *ESCHERICHIA COLI* BY MULTIPLE ANTIBIOTIC RESISTANCE AND RIBOTYPE PROFILES. Salina Parveen* and Mark L. Tamplin, Food Science and Human Nutrition, University of Florida, Gainesville, FL 32611.

Estuarine waters receive fecal pollution from a variety of sources, including human and wildlife. *E. coli* is one of several fecal coliform bacteria that inhabit the intestines of warmblooded animals. Methods are required to specifically differentiate sources of pollution, which impact estuaries, and influence remediation efforts. A total of 765 *E. coli* isolates from point (PS) and non-point (NPS) sources were collected from the Apalachicola National Estuarine Research Reserve and tested for multiple antibiotic resistance (MAR) profile using a total of 10 antibiotics. *E. coli* from PS showed significantly greater resistance ($p = <0.05$) to antibiotics and higher MAR indices than NPS isolates, except for penicillin G. Sixty-five different resistance patterns were observed among PS isolates, compared to 32 for NPS isolates. Profile homology based on coefficient of similarities showed that PS isolates were more diverse than NPS isolates. Ribotype was also determined for selected isolates. It was found that PS isolates showed less diversity in profile. *E. coli* isolates were also obtained directly from human and animal feces, and showed high homology in MAR and RT profiles with PS and NPS isolates, respectively. We conclude that MAR and RT profiles may be a useful method to identify sources of fecal pollution within estuaries and facilitate management practices.

OZONE ASSISTED DEPURATION OF RED TIDE CONTAMINATED SHELLFISH. Gary E. Rodrick,* Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL 32611-0370.

Ozone treatment of seawater may serve as a means to detoxify or inactivate many toxins of marine origin that are of public health importance. For example, *Gymnodinium breve*, the red tide dinoflagellate contains potent toxins that have been associated with massive fish kills, other animal mortalities such as manatees and human morbidity in the Gulf of Mexico. If present in large enough quantities, the toxins can be concentrated in the tissues of various molluscan shellfish making them toxic for human consumption. Blooms of red tide have occurred near Apalachicola Bay and attempts to recover the contaminated resource through depuration with ultraviolet light and chlorination have proven unsuccessful.

However, ozone assisted depuration experiments have been successful in both Europe and Australia.

Results from our laboratory indicate that ozone can be used to effectively kill the red tide organism and also inactivate the associated toxins in seawater. Specifically, *G. breve* toxins were exposed to ozone treatment in both extracted form and intact whole cells. Samples displayed a three log reduction in the total amount of toxin (PbTx-1, 2, 3, 5, 7, and 9) recovered after 10 minutes as determined by HPLC analysis. Ozone effectively killed the red tide dinoflagellates when directly contacted ozone and when exposed in a pre-ozonated ASW environment. Both samples, when examined by light microscopy, displayed little difference between the direct and indirect ozone treatments. Reduction in toxin levels directly correlated with reduction of toxicity as observed using a fish (*Cyprinodon variegatus*) bioassay.

ASSESSING THE RISK OF *VIBRIO VULNIFICUS* IN MOLLUSCAN SHELLFISH. Mark L. Tamplin* and J. Keith Jackson, Institute of Food & Agricultural Sciences, University of Florida, Gainesville, FL 32611-0310.

In the US, *Vibrio vulnificus* is the leading cause of death traced to consumption of raw shellfish. It is normal microflora in marine environments where it persists among oyster microflora as a genetically heterogeneous population. Risk correlates with seasonally high numbers during summer months, when consumers can ingest hundreds of *V. vulnificus* strains per meal. Currently, the infectious dose for humans is unknown, as well as whether disease is caused by single or multiple strains found in molluscan shellfish. In these studies, we found that ca. 10^3 *V. vulnificus*/gram of oyster was associated with human infections, and that a single *V. vulnificus* strain, evidenced by pulsed-field gel electrophoresis profile, was isolated from human tissues. When mice were inoculated with a mixture of strains, only the high virulent strain was isolated from tissues. These data indicate that human *V. vulnificus* infections may be initiated by multiple strains with variable virulence, but that later stages of disease result from strains that show high virulence in mice. Environmental levels exceeding 1,000/g may be considered hazardous to vulnerable human populations.

GENE CONSERVATION: MANAGEMENT AND EVOLUTIONARY UNITS IN FRESHWATER MUSSEL MANAGEMENT

ALTERNATE MODELS OF GENETIC STRUCTURE IN UNIONID POPULATIONS: CONSERVATION AND MANAGEMENT IMPLICATIONS. David J. Berg, Department of Zoology, Miami University, Hamilton, OH 45011; Walter R. Hoeh and Sheldon I. Guttman, Department of Zoology, Miami University, Oxford, OH 45056.

North America is a region of immense freshwater mussel diversity. However, many of the endemic taxa are threatened with

extirpation. To successfully conserve variation within taxa, management agencies must understand the genetic structure of populations. We used allozyme electrophoresis to characterize partitioning of genetic variation within-populations (w-p) and among populations (a-p) of unionids in the Ohio River system and within the Big Darby Creek system of central Ohio. *Quadrula quadrula* typically occupies large rivers, while *Elliptio dilatata* is a common resident of small streams such as Big Darby Creek. On average, populations of *Q. quadrula* contained greater w-p variation (2.1 alleles/locus, 61% polymorphic loci, 24% heterozygosity) than populations of *E. dilatata* (1.6, 32%, 10%, respectively). Patterns of a-p variation differed between species. Allele frequencies of *Q. quadrula* were not different among populations >1000 km apart. Populations of *E. dilatata* showed differences in allele frequencies between populations <100 km apart. Unionid species illustrate at least 2 models of the partitioning of genetic variation. Model I species such as *Q. quadrula* have a high gene flow among populations; each population contains much of the total variation present within a large geographic region. Model II species such as *E. dilatata* have restricted gene flow and large amounts of a-p variation; individual populations exhibit unique arrays of alleles. Large river habitats are more stable, capable of supporting larger populations of mussels, and may contain fishes with greater dispersal capability than small streams. The result of this combination is a single large metapopulation in big rivers. Preservation of several populations in big rivers will conserve most of a taxon's genetic diversity. Conservation of similar amounts of genetic diversity in small streams will require protection of a large number of populations within any geographic region. Such differences require that management agencies consider the genetic structure of mussel taxa when developing conservation plans.

BIOMARKER ASSESSMENT OF ENVIRONMENTAL CONTAMINATION WITH FRESHWATER MUSSELS.

Marsha C. Black, Environmental Health Science Program, College of Agricultural and Environmental Sciences, The University of Georgia, Athens, GA 30602–2102.

Bivalves are effective pollution biomonitors in marine and freshwater environments because of their ability to bioconcentrate many environmental pollutants to levels that greatly exceed those contained in water and sediments. However, most research efforts have focused on monitoring chemical accumulation by mussels, and have not examined the toxic effects of accumulation or exposure to toxic chemicals. In addition, different phases of the mussel life cycle have been sparingly employed for toxicity evaluation.

A recent focus in environmental toxicology has been the development of biomarkers—rapid, toxicological assays that detect sublethal biochemical, physiological and organismal changes following exposure to chemical contaminants. Biomarkers can be screening tools to detect exposure to environmental contaminants and can also quantify specific toxicological responses in exposed

organisms. Our research has focused on developing biomarker assays for adult and larval freshwater mussels. We have conducted laboratory and in-situ studies with *Anodonta grandis*, *Quadrula quadrula*, *Utterbackia imbecillis* and *Corbicula fluminea* (the Asiatic clam), primarily using DNA strand breakage (an indicator of genotoxicity) and the nonspecific biomarkers, growth and condition index to detect exposure and effects of environmental pollution. Current projects include the development of these biomarkers in newly transformed larval *U. imbecillis* and testing additional biomarker assays on adult and larval mussels exposed to heavy metals, agricultural and urban runoff. Ultimately, biomarker data with mussels will be used to develop exposure and effects assessment protocols for use in risk assessments.

MANAGEMENT UNITS AND EVOLUTIONARY SIGNIFICANT UNITS IN CONSERVATION. **Brian W. Bowen**, Dept. of Fisheries and Aquatic Sciences, University of Florida, Gainesville, FL 32611.

As biological information is translated into conservation policy, scientists are challenged to “get real,” to transform previously intangible concepts into quantifiable terms. A prominent example of this process is the need to define taxonomic units in a legal context, rekindling a debate over species concepts. Another example of applied biology is the need to objectively define conservation priorities. Such priorities are inevitable when resources applied to conservation are dwarfed by the scope of environmental degradation. One relatively new approach to these issues is to circumvent the sticky questions surrounding species definitions and consider endangered biota as management units (MU: demographically independent populations) or evolutionary significant units (ESU: lineages with unique genetic attributes). While both categories may be afforded protection under the Endangered Species Act and similar legislation, the ESU may represent a larger proportion of biodiversity and hence a stronger candidate for protection. In most circumstances these categories seem to provide an acceptable yardstick for assigning conservation priorities. The exceptions are the cases of incipient speciation, where lineages may have evolved unique life-history attributes, but have not diverged sufficiently to be considered as ESUs. Since these groups may be the progenitors of future biodiversity (future species), organisms that straddle the boundaries of MU and ESU merit careful deliberation in assigning conservation priorities.

CORRELATION BETWEEN MATING SYSTEM AND DISTRIBUTION OF GENETIC VARIATION IN *UTTERBACKIA* (BIVALVIA: UNIONIDAE). **Walter R. Hoeh** and **Sheldon I. Guttman**, Department of Zoology, Miami University, Oxford, OH 45056; **David J. Berg**, Department of Zoology, Miami University, Hamilton, OH 45011.

Variation in mating systems (*i.e.*, self-fertilization vs. cross-fertilization) has been shown to affect the distribution of genetic

variation in plants. However, the paucity of this type of variation in closely related taxa has hampered similar evaluations in animals. Comparisons of the level of within- and among-population allozymic variation in the simultaneous hermaphrodite, *Utterbackia imbecillis*, with those of the gonochoric (=dioecious) *U. peggyae* and *U. peninsularis* allowed inferences to be made regarding 1) the mating system of *U. imbecillis* and 2) the population genetic structure of these three species. The low levels of within-population variation and marked heterozygote deficiency observed in *U. imbecillis*, relative to that observed in *U. peggyae* and *U. peninsularis*, suggest that there is a high degree of self-fertilization in *U. imbecillis*. However, the among-population variation in the level of heterozygote deficiency is consistent with the hypothesis that the relative amounts of cross-fertilization and self-fertilization vary among populations of *U. imbecillis*. The hypothesis of high levels of self-fertilization in *U. imbecillis* is consonant with the presumed high colonization potential of this species. The estimates of F_{st} obtained for the three species of *Utterbackia* suggest a very high level of among-population genetic differentiation (F_{st} range: 0.563–0.821). This observation is quite unexpected for an able colonizer such as *U. imbecillis*. This result suggests that high F_{st} values are not restricted to species with relatively low colonizing potential. Furthermore, the efficacy of inferring levels of gene flow from F_{st} estimates may be severely compromised in species with high levels of self-fertilization.

GENETIC DIVERSITY AMONG SEVERAL SPECIES OF UNIONID MUSSELS IN ARKANSAS. Ronald L. Johnson,* Fang Qing-Liang, and Jerry L. Farris, Arkansas State University, Department of Biology, State University, AR 72467.

Allozyme analysis was utilized to determine the genetic diversity of 319 individuals for four species of mussels (*Amblema plicata*, *Plectomerus dombeyanus*, *Quadrula pustulosa*, and *Q. quadrula*) in the Cache and White Rivers of Arkansas. Mussel populations of both rivers are subjected to frequent harvest, while White River populations are exposed to periodic habitat destruction due to dredging. Nine enzyme systems representing sixteen loci were selected for analyses based upon their expression in adductor muscle. Species of the Cache River exhibited the greatest polymorphism (P), yet heterozygosity (H) values between rivers were inconsistent. Ranges of P were from 0.572 for *A. plicata* to 0.360 for *Q. quadrula*; H values ranged from 0.049 for *P. dombeyanus* to 0.144 for *Q. pustulosa*. H and P values of the Amblemini of the Cache and White Rivers were consistent both in historical context and genetic diversity with those of previous studies. Populations were characterized by heterozygote deficiencies at all loci. Several determinants of heterozygote deficiency were investigated, with selection chosen as the probable mechanism. Although there are no visible signs of genetic decline associated with bottlenecking in the present study, mussel beds are on the decline in Arkansas, and loss of genetic diversity is detrimental to the stability of populations.

CLARIFICATION OF PLEUROBEMA PYRIFORME AS A SPECIES OR SPECIES-COMPLEX AND IMPLICATIONS FOR THE CONSERVATION OF RARE FRESHWATER MUSSELS. Karen L. Kandl,* Hsin-Ping Liu, and Margaret Mulvey, Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29802; Robert Butler, U.S. Fish and Wildlife Service, Asheville Field Office, Asheville, NC 28801; W. Randy Hoeh, Miami University, Department of Zoology, Oxford, OH 45056.

Pleurobema pyriforme is currently a species-complex that includes *P. pyriforme* (oval pigtoe), *P. bulbosum* (inflated clubshell), and *P. reclusum* (Florida pigtoe). *P. pyriforme* occurs from the Apalachicola River system to the Suwannee river system although some researchers consider it to be an endemic of the Apalachicola River system. The historic range of *P. bulbosum* included the Flint, Chipola, and Ochlockonee River systems, and the range of *P. reclusum* includes the Ochlockonee and Suwannee River systems. Morphological evidence suggests that *P. bulbosum* and *P. reclusum* should be considered species distinct from *P. pyriforme*. One other species, *P. strodeanum* (fuzzy pigtoe), is currently recognized in this region; its range extends from the Escambia River to the Choctawhatchee River system. We are amplifying a 2.2 kb DNA fragment of the ITS region and cutting this with nine restriction enzymes. The resulting polymorphisms (RFLPs) are used to examine the genetic relationship among these taxa. Molecular genetic data, in addition to morphological evidence, can help clarify the status of putative species. Because these taxa are rare or endangered, the clarification of the status of these mussels is especially important to their conservation.

GEOGRAPHIC SCALE AND MOLECULAR STOCK ASSESSMENT. Stephen A. Karl, Department of Biology, University of South Florida, Tampa, FL 22620–5150.

Ecologically and commercially important aquatic species often are dispersed over large geographic areas. The management of such species frequently relies on a Geo-political Species Concept (GSC) to designate the limits to significant biological units. In a GSC, recognized political or geographic boundaries serve as surrogates to true biological or evolutionary processes limiting dispersal. Species and subsequent management units are defined primarily by abiotic factors assumed to reflect true biological entities. Although the GSC is expedient and has some utility in conservation schemes, several factors conspire to remove the efficacy of this approach. Primary among the confounding attributes of a GSC is the inability to determine, *a priori*, a biologically appropriate geographic scale for the species. Commonly, aquatic invertebrates are characterized by a functionally sessile adult possessing a plankton dispersing larvae and can pose a significant challenge to a GSC. Here, I discuss issues concerning the role of geographic scale in identifying biologically appropriate stocks. Example from deep-sea hydrothermal vent clams and tube worms and the Eastern Oyster will serve to illustrate how *a priori* assumptions concerning

larval dispersal ability and geographic scale can be positively misleading identifiers of biological stocks.

MOLECULAR PHYLOGENETIC RELATIONSHIPS AMONG FRESHWATER MUSSELS OF THE SUBFAMILY ANODONTINAE: CONSERVATION IMPLICATIONS.

Hsin-Ping Liu and Margaret Mulvey, Savannah River Ecology Laboratory, University of Georgia, Drawer E, Aiken, SC 29802.

Anodontinae exhibit an array of conchological, anatomical, life history and reproductive characters. This array of interspecific variability invites evolutionary explanations. A well-supported phylogenetic relationship of the Anodontinae is essential to test hypotheses of evolutionary processes. Historically, there has been strong reliance on conchological characters for species recognition. However, conchological characters are notoriously variable; morphological variation can be great within populations, as well as among populations and species. This variation may reflect phenotypic plasticity or evolutionary convergence; whatever the underlying cause, the effect is to make species recognition contentious even among experts. The purposes of this study are: (1) to use mitochondrial cytochrome oxidase subunit I (COI) sequence data to assess relationships among Anodontinae species, and (2) to use this phylogenetic reconstruction to examine the evolution of life history and reproductive traits.

SPECIES AND SUBSPECIES: PROTECTING AQUATIC INVERTEBRATES UNDER THE ENDANGERED SPECIES ACT OF 1973, AS AMENDED. Ren Lohofener, Fish and Wildlife Service, Arlington, VA.

The Endangered Species Act of 1973, as amended, provides protection for species and subspecies of invertebrates. For vertebrates, populations can also be protected. The subjectivity in defining what constitutes a subspecies can lead to difficulties in defending the taxonomic designation during the listing process. Because of the difficulty in defining what constitutes a subspecies, and the inability to extend the Act's protection to invertebrate populations, using molecular genetic techniques to help define and defend recognition of species and subspecies will help gain protection for threatened or endangered aquatic invertebrates.

Recognition of imperiled taxa is the first step in recovery. Ideally, identification of "species" (including subspecies) in decline will occur before the species' condition has deteriorated to the point of needing the Act's protection. Protection of the species through candidate conservation measures may be more effective than recovery of species once they have been listed: it should take less time and money to conserve a candidate species and partners may be more willing to participate in the conservation process.

If the species declines to the point of needing the Act's protection, the recovery process begins immediately. A recovery out-

line defines the major tasks and partners needed to recover the species. These tasks are implemented as soon as funding and developing partnerships permit. During the course of recovery, Section 7 consultations with federal agencies and Habitat Conservation Plans with the private sector, prevent activities that would jeopardize the species. "Safe Harbor Agreements" with private landowners benefit the species while providing assurances to landowners. While it is certainly best for the species (and the ecosystem) to conserve species before they need to be listed, the Act is an effective safety net to prevent extinction.

THE ROLE OF NATIONAL WILDLIFE REFUGES IN CONSERVING THE BIOLOGICAL AND GENETIC DIVERSITY OF FRESHWATER MUSSELS. Patricia A. Morrison, U.S. Fish and Wildlife Service, Ohio River Islands National Wildlife Refuge, P.O. Box 1811, Parkersburg, WV 26102-1811.

The National Wildlife Refuge System is a network of over 500 refuges comprising over 90 million acres managed for the conservation and enhancement of fish and wildlife and their habitats. Two system-wide objectives directly relate to freshwater mussels: "to preserve, restore, and enhance in their natural ecosystem (when practicable) all species of animals and plants that are endangered or threatened with becoming endangered," and "to preserve a natural diversity and abundance of fauna and flora on refuge lands." Nearly 60 species of freshwater mussels are currently federally endangered or threatened, and many are feared extinct. Many refuges are now actively managing freshwater mussels—not only conducting much needed systematic inventories, but also participating in important research projects, habitat restoration, and mussel reintroduction programs. Few refuges have good information on historic and present mussel diversity, and some are actively working toward restoration of mussel communities. However, little attention has been paid so far to genetic considerations, probably due to a lack of information identifying distinct populations. Refuges can contribute significantly to the conservation of freshwater mussel fauna by preserving mussel communities in place, providing areas for important research, and participating in genetics repository work.

GENETIC RELATIONSHIPS AMONG ATLANTIC SLOPE LANCEOLATE *ELLIPTIO*: RFLPS OF AMPLIFIED ITS REGION AND ALLOZYMES. Margaret Mulvey and Hsin-Ping Liu, Savannah River Ecology Laboratory, University of Georgia, Drawer E, Aiken, SC 29802.

Lanceolate *Elliptio* group are defined as having shell height to shell length ≤ 0.43 . Johnson (1970) viewed this variation as phenotypic plasticity and has lumped all lanceolate *Elliptio* under *E. lanceolata* (with 20 synonyms) or *E. shepardiana*. Davis (1984) using allozymes showed that there are at least five genetically

discrete lanceolate species and argued that conchological similarities reflect convergent evolution.

In this study we are concerned with the following questions. How useful are restriction fragment length polymorphisms (RFLPs) of the ITS region for discriminating among species that have been described primarily or exclusively on the basis of conchological characters? Can we obtain insight into patterns of speciation in the lanceolate *Elliptio* group? The following are our results.

1. RFLPs of ITS region are useful to differentiate *Elliptio* species.
2. Both RFLPs and allozyme data showed that there are at least six discrete lanceolate species (*E. angustata*, *E. fisheriana*, *E. folliculata*, *E. lanceolata*, *E. producta*, and *E. shepardiana*) on the Atlantic slope.
3. Names currently applied to some shell phenotypes are not consistent with genetic data.

NMFS AND THE EVOLUTIONARILY SIGNIFICANT UNIT CONCEPT FOR PACIFIC SALMON. Marta Nam-mack, National Marine Fisheries Service, 1315 East-West Highway, Silver Spring, MD 20910.

The National Marine Fisheries Service (NMFS) published a policy on applying the definition of species under the Endangered Species Act (ESA) to Pacific salmon on November 20, 1991 (56 FR 58612). This policy states that a stock of Pacific salmon will be considered a distinct population, and hence a "species" under the ESA, if it represents an evolutionarily significant unit (ESU) of the biological species.

NMFS has been implementing this policy for Pacific salmonids since it listed Snake River sockeye salmon in 1991. While genetic data play a central role in NMFS' ESU concept, they are not always available, and NMFS makes an effort to compile and evaluate available phenotypic, life history, and habitat information when conducting status reviews. Examples of how NMFS has used the ESU policy to delineate ESUs are provided.

NMFS and the U.S. Fish and Wildlife Service (FWS) issued a joint policy on the recognition of distinct vertebrate population segments under the ESA on February 7, 1996.

The concept of a distinct population segment can be applied to invertebrates in proactive efforts to protect species outside of the ESA. As Waples (1995) states, "Outside the ESA, conservation efforts might be guided by any of several alternative contexts for interpreting evolutionary significance . . . The key factor is how conservative one wants to be (or can afford to be) in attributing evolutionary significance to a biological unit." Genetic, phenotypic and life history data, and habitat characteristics can provide valuable information to managers of invertebrate species on which units to conserve in order to prevent ESA listings and recover species.

SPECIES DELINEATION AND THE IDENTIFICATION OF EVOLUTIONARILY SIGNIFICANT UNITS IN THE FRESHWATER MUSSEL GENUS *POTAMILUS* (Bivalvia: Unionidae). Kevin J. Roe* and Charles Lydeard, Aquatic Biology Program, Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487-0344.

Confidence in the identification of species within many unionid genera has been thwarted by a combination of an overall conservative bauplan coupled with a high degree of ecomorphological variation. The identification of "real" species or evolutionarily significant units (ESU's) is deemed essential to effective management and conservation of extant freshwater mussel taxa. DNA sequence data have the potential to allow for the identification of ESU's which would otherwise go unnoticed (cryptic species). In conducting a phylogenetic study of the genus *Potamilus* based on DNA sequences of a portion of the mitochondrial cytochrome oxidase I gene; we have identified two genetically distinct populations of the threatened inflated heelsplitter (*Potamilus inflatus*). We feel the high level of genetic difference between these populations relative to other congeners warrants their recognition as distinct species. The conservation implications of this recognition are discussed.

TRANSLOCATION PROGRAMS IN FRESHWATER MUSSELS: GENETIC AND DISEASE CONCERNS. Rita Villella and Tim King, U.S. Geological Survey-Biological Resources Division, Leetown Science Center, Aquatic Ecology Laboratory, 1700 Leetown Road, Kearneysville, WV 25430; Cliff Starliper, U.S. Geological Survey-Biological Resources Division, Leetown Science Center, National Fish Health Research Laboratory, 1700 Leetown Road, Kearneysville, WV 25430.

The diverse and abundant freshwater mussel fauna of North America is declining at a rate not experienced by other faunal groups. The recent introduction of the exotic zebra mussel (*Dreissena polymorpha*) is seen as a major threat to an already declining and threatened fauna and has resulted in State and Federal resource agencies attempting to mitigate these impacts through salvage and relocation efforts. Relocation of mussels has been used as a conservation tool to enhance native mussel populations, move them from areas of high zebra mussel infestations, and to re-establish populations of endangered species or populations previously eliminated by pollution or habitat degradation. The primary concerns of conservation efforts, such as salvage programs and translocations, should be conservation of the gene pool and prevention of disease transmission. It is imperative that geographic populations of species targeted by salvage and translocation programs be segregated in holding facilities until such time the genetic structure and effective population sizes are determined. This precaution is necessary to prevent the occurrence of outbreeding and inbreeding depression and hybridization. The potential conse-

quences of pathogen and disease contagion that might be associated with relocation of native molluscs parallels the concerns for genome conservation. Aspects of this include the effect on the relocated animals as well as those species of mussels or fish present in the receiving waters including other natural environments and hatchery facilities used for maintenance and propagation. We will discuss these concerns and provide recommendations for avoiding genetic- and disease-related losses of the mussel resources the salvage and relocation programs aspire to conserve.

NATIONAL STRATEGY FOR THE CONSERVATION OF NATIVE FRESHWATER MUSSELS. Susi von Oettingen, U.S. Fish and Wildlife Service, 22 Bridge Street, Concord, NH 03301–4986; Debbie Mignogno, U.S. Fish and Wildlife Service, 300 Westgate Center Drive, Hadley, MA.

The continental United States contains the world's greatest diversity of freshwater pearly mussels, nearly 300 species. This faunal group has been characterized as 6 percent extinct, 19 percent threatened or endangered, and 23 percent as potentially warranting Federal protection. No other widespread group of animals in North America approaches this level of faunal collapse. At an April 1995 meeting of representatives from several Federal and State natural resources agencies and the commercial mussel industry, the magnitude and the immediacy of threats, nationwide, to our native freshwater mussel fauna was recognized. The group agreed that a coordinated effort of national scope was needed to prevent further mussel extinctions and population losses. To address these needs, the group decided to: 1) draft a National Strategy for the Conservation of Native Freshwater Mussels; and 2) establish a national ad hoc committee with broad based representation from State, Tribal and Federal agencies, the mussel industry, private conservation groups, and the academic community to help implement mussel conservation at the national level. A draft National Strategy was presented in October 1995 at a national mussel symposium in St. Louis, Missouri. I will discuss the strategy and the results of the first ad hoc committee meeting scheduled for February 1997.

CONSERVATION STATUS OF FRESHWATER MUSSELS: FAMILIES MARGARITIFERIDAE AND UNIONIDAE. James D. Williams, U.S. Geological Survey, Biological Resources Division, 7920 NW 71 Street, Gainesville, FL 32653.

The United States has the greatest diversity of freshwater mussels in the world, nearly 300 species and subspecies. The decline of mussels has gone almost unnoticed due to insufficient inventory and monitoring and research on biology and conservation. Decline of freshwater mussels has resulted from a variety of habitat perturbations. The most significant impacts have come from habitat destruction. Competition from non-native mollusks, the Asian

clam, *Corbicula fluminea* and zebra mussel, *Dreissena polymorpha*, has also contributed to the loss of native mussels. Of the 297 native mussel species, 213 (71.7%) are considered endangered, threatened, or of special concern. This figure includes 21 mussels (7.1%) that are endangered and presumed extinct. Only 70 species (23.6%) are considered to have stable populations. Future trends in molluscan extinction will depend on the nation's ability to change the direction taken in conservation and recovery of endangered species. Conservation and restoration efforts should be focused on ecosystems and watersheds, instead of individual species. To reverse the current rate of species loss, a stronger commitment from state and federal agencies and increased public involvement will be required.

MARINE GENETICS

SELECTION OF OYSTERS FOR RESISTANCE TO TWO PROTOZOAN PARASITES. Lisa M. Ragone Calvo,* Valerie Harmon, and Eugene M. Burreson, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

The application of classic agricultural selective breeding strategies in the field of aquaculture has resulted in enhanced performance of various finfish and shellfish species. This approach has been successful in producing strains of oysters, *Crassostrea virginica*, that are resistant to the protozoan parasite *Haplosporidium nelsoni*; however, little resistance to a second pathogen, *Perkinsus marinus*, was conferred limiting the utilization of these strains. Since 1988 we have been selecting oysters for resistance to both parasites. Oysters surviving 2 to 3 years of exposure to the pathogens have been used to produce F1–F3 strains. Three strains were evaluated from 1993–1995—a Delaware Bay F3, a lower James River F3, and a Louisiana F1 strain. Oysters were spawned in April 1993 and deployed in the lower York River, VA in August 1993. Growth, mortality, and disease were subsequently monitored through December 1995.

Both parasites were abundant during all three years of the study. The Delaware Bay strain performed exceptionally well and showed significantly higher survival and growth than either the James River or Louisiana strain. By December 1994, mean shell height of the Delaware Bay strain was 74 mm, nearly 90% of the oysters were market size (64 mm), and mortality was only 16%. In contrast, mortalities exceeded 40% and mean shell heights were below market size in the two other strains. During the first two years, the Delaware Bay strain was more resistant to *H. nelsoni* than the other strains but had higher prevalences of *P. marinus*. Infection levels were more variable between groups the third year and final cumulative mortalities were 53% in the Delaware strain, 75% in the Louisiana strain, and 83% in the James River strain.

Compared to susceptible wild oysters all three strains exhibited decreased disease susceptibility. These results indicate that resistance to both *P. marinus* and *H. nelsoni* can be achieved through selective breeding.

AMPLIFICATION AND SEQUENCING OF THE *BONAMIA OSTREAE* 18S rDNA GENE: PHYLOGENETIC CONSIDERATIONS AND APPLICATIONS. Ryan B. Carnegie,* School of Marine Science, University of Maine, Orono, ME 04469; Daniel L. Distel, Department of Biochemistry and Molecular Biology, University of Maine, Orono, ME 04469; Bruce J. Barber, School of Marine Science, University of Maine, Orono, ME 04469.

The eukaryotic 18S rDNA gene has found a range of uses in the study of marine protistan parasites. The conserved nature of this gene has allowed phylogenetic comparisons among distantly related taxa, and hypervariable regions within the gene have been used to design specific probes and polymerase chain reaction (PCR) primers. We have isolated and sequenced the putative 18S rDNA gene of *Bonamia ostreae*, the intrahemocytic parasite of the European flat oyster, *Ostrea edulis*. After purifying genomic DNA from *B. ostreae*-enriched oyster hemolymph, we amplified a sequence including ~400 bp at the 3' terminus of the 18S rDNA gene and the adjacent internal transcribed spacer (ITS1) region using eukaryotic, universal PCR primers. Amplicons of ~800 bp and ~600 bp were produced, the first being identical to *O. edulis* 18S/ITS1. We found that the second amplicon included a unique, protistan, partial 18S gene, and designed a PCR primer from a unique region to be used with a universal primer to amplify the remainder of this gene. The sequence we have obtained will be verified as belonging to *B. ostreae* using fluorescent *in situ* hybridizations.

Initial comparisons of this sequence with other protistan sequences revealed a similarity (85%–89%) to the dinoflagellates of the Orders Gonyaulacales and Gymnodiniales. Sequence similarity to *Haplosporidium nelsoni*, by comparison, was 76%. These data suggest that *B. ostreae* may, like *Perkinsus* spp., share an affinity closer to the dinoflagellates than the Haplosporidia or Apicomplexa. We will investigate these phylogenetic relationships, and discuss the development of a sensitive and specific molecular probe for the diagnosis of bonamiasis in European oysters.

GENETIC SELECTION IN OYSTERS FOR GROWTH AND RESISTANCE TO JUVENILE OYSTER DISEASE (JOD). Christopher V. Davis,* Maya A. Croshy, Bruce J. Barber, and Robert O. Hawes, Department of Animal, Veterinary and Aquatic Sciences, University of Maine, Orono, ME 04469.

Juvenile Oyster Disease (JOD) is a relatively recent phenomenon adversely affecting growers of Eastern oysters (*Crassostrea virginica*) in the northeastern United States. In Maine, JOD mortalities have resulted in juvenile crop losses exceeding 90% with equally high mortalities in the other northeastern states. Ongoing research has largely focused on determining the causative agent of

the disease, but use of genetically selected broodstock and specific management options may help alleviate the problem for growers. The goals of this project were to determine the size specificity, temporal and spatial variability of JOD outbreaks with respect to several genetic lines. A genetically selected line (including a within line unselected control) having undergone three generations of selection for faster growth along with an unselected wild line were deployed at two growing sites historically impacted by JOD. Biweekly monitoring of size (shell height and live weight) and mortality of replicate cohorts over the first growing season allowed the investigators to track oyster growth and incidence of JOD mortality. Onset of JOD symptoms at both study sites occurred 4–6 weeks later than has been typically seen in recent years. Significant differences in growth and JOD induced mortality were observed both within and among genetic lines at both growing locations. These results suggest that selective breeding for growth and disease resistance can greatly benefit oyster growers plagued by JOD.

ATTEMPTED HYBRIDIZATION BETWEEN THE PACIFIC AND AMERICAN OYSTERS BY UNBALANCED GENOMIC COMBINATIONS. Xining Guo and Standish K. Allen, Jr.,* Haskin Shellfish Research Laboratory, Rutgers University, 6959 Miller Avenue, Port Norris, NJ 08349; Zhaoping Wang, College of Fisheries, Qingdao Ocean University, Qingdao, Shandong 266003, PRC.

The transfer of disease resistance from the Pacific oyster (*Crassostrea gigas*) to the American oyster (*C. virginica*) is prohibited by post-gametic barriers to hybridization, which may be caused by only a small number of incompatible genes and potentially bypassed with unbalanced combinations of the two genomes. In this study, we attempted to hybridize the two oysters with a wide range of aneuploid and polyploid combinations of their genomes. Seven types of hybrids were produced between the two oysters with various genomic combinations: 1) normal diploid hybrids with a genomic combination of 1 + 1; 2) 3n hybrids with a genomic combination of 2 + 1, produced by blocking polar body II in a normal hybrid cross; 3) 2n–3n aneuploid hybrids with a 1 + 1.5 genome produced by mating diploids with triploids; 4) 3n ± aneuploid hybrids with a 1.5 + 1.5 genome produced by mating triploids with triploids; 5) 3n–4n aneuploid hybrids with a 2 + 1.5 genome produced by blocking polar body II in diploid female × triploid male crosses; 6) 2n–5n aneuploid hybrids with a (1 – 4) + 1 genome produced by blocking polar body I in diploid crosses; 7) 2n–7n aneuploids with a (1.5 – 6) + 1 genome produced by blocking polar body I in triploid female × diploid male crosses. Each group was replicated 3–6 times. At 24 hours post-fertilization, all groups produced significant numbers of D-stage larvae. However, no larvae survived beyond three weeks of age (except intraspecific controls), suggesting that those unbalanced genomic combinations were not capable of breaking the hybridization barriers between the two oyster species.

THE PORTUGUESE OYSTER *CRASSOSTREA ANGULATA* IS OF ASIAN ORIGIN. Diarmaid O'Foighil, Museum of Zoology and Department of Biology, University of Michigan, Ann Arbor, MI; Patrick M. Gaffney,* College of Marine Studies, University of Delaware, Lewes, DE 19958; Thomas J. Hilbish, Department of Biology, University of South Carolina, Columbia, SC 29208.

For years oyster biologists have debated the taxonomic status of the Portuguese oyster, *Crassostrea angulata* (Lamarck). Many have argued that Portuguese oysters are in fact Pacific oysters, *C. gigas* (Thunberg). An impressive list of similarities between the two taxa includes larval shell morphology, enzyme polymorphisms, chromosomal karyotype, and the apparent ability to interbreed.

If Portuguese and Pacific oysters are the same or very closely related species, how did their disjunct distribution come about? One hypothesis is that *C. gigas* originated in the Atlantic and was introduced into Japan during the 16th century by Portuguese traders. Alternatively, Atlantic populations of *C. angulata* may be descendants of Pacific oysters first brought to Portugal by the same traders.

The application of molecular phylogenetic methods by several investigators has confirmed the Asian affinities of the Pacific oyster. The remaining question is whether the Portuguese oyster is in fact a recent isolate of *C. gigas*.

We present a phylogenetic analysis based on mitochondrial DNA (16S rDNA and cytochrome oxidase I) sequence data. Our results show the Portuguese oyster to be closely related but not identical to present-day *C. gigas* from Japan. The ancestral population that gave rise to *C. angulata* may no longer exist in Japan; alternatively, *C. angulata* may be derived from *C. gigas* from another part of Asia.

EVALUATION OF AMERICAN OYSTER STOCKS: DISEASE RESISTANCE AND GENETICS. Kennedy T. Paynter, Jr., University of Maryland, College Park, MD 20742; Patrick M. Gaffney,* College of Marine Studies, Lewes, DE 19958; Donald W. Meritt, Horn Point Environmental Laboratories, University of Maryland, Cambridge, MD 21613.

The American oyster *Crassostrea virginica* inhabits coastal and estuarine environments from Canada to Mexico, spanning a tremendous range of environments and several biogeographical boundary zones. If the species is divided into genetically distinct subpopulations, it seems likely that they will exhibit differences in important quantitative traits such as growth rate, disease resistance, and survival in particular environments.

By analysis of mitochondrial DNA sequence variation, we have found that in addition to the Gulf Coast vs. Atlantic phylogeographic break already reported, Atlantic *C. virginica* form three discrete entities: South Atlantic, North Atlantic, and Canadian Maritime populations. In view of this genetic subdivision of the species, we are investigating the performance of hatchery lines of

different genetic origins in a common-garden experiment. Five lines were constructed in 1995, using a common pool of eggs from twelve Chesapeake Bay (CB) females. Each line was formed by combining an aliquot of the CB egg pool with pooled sperm from five males of common geographic origin: Delaware Bay, North Carolina, South Carolina, Louisiana and Texas. In addition, a CB \times CB line was produced using different parents. These lines, along with additional lines from Louisiana, Oregon and North Carolina were deployed in 1995 at three Chesapeake Bay sites: the Chester and Choptank Rivers and Mobjack Bay. Oysters at the two low-salinity show negligible disease-related mortality. In Mobjack Bay, we observed significant differences among lines in disease prevalence and mortality, indicating that some populations may be superior as sources for broodstock for initiating selective breeding programs.

PHYLOGENETIC ANALYSIS OF THE HAPLOSPORIDIA BASED UPON ACTIN GENE SEQUENCES. Kimberly S. Reece,* Mark E. Siddall, and Eugene M. Bureson, School of Marine Science, Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062.

Members of the phylum Haplosporidia include a variety of parasitic protozoans of marine and freshwater invertebrates. Historically Haplosporidia have been a troublesome group for taxonomists and phylogeneticists resulting in numerous proposals of taxonomic schemes. Proper placement of the group within the protists, as well as within-phylum relationships of these parasites, remains controversial. Molecular analyses based upon the small subunit ribosomal RNA (SSU rRNA) gene sequences have supported a phylogenetic affinity of the haplosporidians with the aveolates (dinoflagellates, ciliates and apicomplexans) although results have been ambiguous regarding a closer affinity to the ciliates or dinoflagellates. Also, these analyses have not supported monophyly of the genus *Haplosporidium* and suggest that current diagnostic criteria for taxonomy are insufficient for accurately distinguishing genera. We have obtained another source of molecular phylogenetic information for several haplosporidians, specifically a 622 bp fragment of actin genes amplified by the polymerase chain reaction. Actin sequences have been obtained from *Haplosporidium nelsoni*, *Haplosporidium louisiana*, *Minchinia chitonis* and *Urosporidium crescens*. The combined information from two differently constrained genes, SSU rRNA and actin, provides for a more robust estimate of haplosporidian relationships.

MITOCHONDRIAL DNA VARIATION AND POPULATION STRUCTURE OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS*. Ami E. Wilbur* and Patrick M. Gaffney, College of Marine Studies, University of Delaware, Lewes, DE 19958.

Geographic variation in morphology and physiology has led to the recognition of three subspecies of bay scallops, and a fourth

has recently been proposed. The hypothesis of restricted gene flow among subspecies was investigated by a genetic survey of scallops from Massachusetts (MA), North Carolina (NC), Florida (FL) and Texas (TX). Sequence variation in two PCR amplified mtDNA regions (16S rRNA and part of the COIII/ATPase coding region) was initially assessed indirectly using RFLP analysis. Results from the RFLP analysis suggested significant variation among populations in haplotype frequencies ($P < 0.001$, Monte Carlo simulation). Average nucleotide divergence among populations, however, was modest (0.7%) and the pattern of divergence among populations was inconsistent with expectations based on geographic proximity. The RFLP approach to describing the genetic relationships of these populations was likely confounded by the highly variable nature of the COIII/ATPase fragment, which resulted in 30 haplotypes in 76 individuals assayed. We have subsequently sequenced the 16S rRNA PCR product from these same populations in an effort to clarify the genetic structure of this species.

MOLLUSCAN DISEASE I

DETECTION AND CHARACTERIZATION OF SUPEROXIDE DISMUTASE ACTIVITY IN *PERKINSUS MARINUS*.

Hafiz Ahmed,* Julie D. Gauthier, Anita C. Wright, and Gerardo R. Vasta. Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E. Pratt Street, Columbus Center, Baltimore, MD 21202.

Perkinsus marinus, an apicomplexan parasite, is known to cause the mass mortalities of oysters (*Crassostrea virginica*) in the Chesapeake Bay. The mechanism of the pathogenesis still remains unclear. For several host-parasite systems, the superoxide dismutases (SODs) are believed to constitute potential virulence factor since they abrogate the host phagocytic oxidative burst. Analysis of cDNA from *P. marinus* has indicated that at least two SODs are expressed. SOD activity was detected in cell extracts by inhibition of pyrogallol auto-oxidation. On non-denaturing polyacrylamide gel electrophoresis and Isoelectric focusing, we have detected multiple bands with SOD activities from *in vitro*—propagated *P. marinus*. Most of the SOD activities were resistant to potassium cyanide, but sensitive to hydrogen peroxide which would be characteristic of FeSOD. The purification of FeSOD and its molecular properties will be discussed.

TREATMENT OF *PERKINSUS MARINUS*-CONTAMINATED MATERIALS. **David Bushek, Russell Holley, and Megan Kelly,** Baruch Marine Field Laboratory, University of South Carolina, Georgetown, SC 29442.

The protozoan oyster parasite *Perkinsus marinus* is a major problem for oyster management and restoration. Transport of

infected oysters or parasite cultures for commerce, habitat management or research, represents a potentially important transmission vector. We examined chlorination, osmotic shock, heat and desiccation as methods to kill cultured *P. marinus* and *P. marinus* in oyster tissues prior to disposal or movement of such material.

Parasites were exposed to various chemical and physical treatments. Viability was determined by uptake of neutral red dye (cultured cells) or parasite enlargement in RFTM (parasites in tissue). About 300 ppm Cl_2 was required to kill all *in vitro* cultured parasites within 30 minutes. Chlorine tolerance was significantly greater in culture medium than seawater, where lower concentrations (52.5 ppm and 170 ppm) were effective with longer exposure times (4 to 18 hrs). Fresh water killed cultured parasites within 30 minutes at room temperature. Temperatures $>40^\circ\text{C}$ killed cultured parasites in seawater or culture medium within 1 hr, but about 50% survived 30 min at 40°C . Parasites within tissues survived 2100 ppm chlorination, fresh water and temperatures of 40 and 50°C for 18 hrs, but were killed within 1 hr at 60°C . After three days of desiccation at room temperature, parasite viability decreased to near zero, but some parasites still responded to incubation in RFTM after 7 days.

Chlorination, osmotic shock, heat, and desiccation can be used to kill *P. marinus*. Parasites within oyster tissues require more extreme treatment. Which method is best for a particular application will depend not only on the state of the parasite (*in vitro* or *in vivo*), but also on available equipment, quantity of the contaminated material, and the economics involved.

MICROPLATE ELISA ASSAY FOR DETECTION OF *PERKINSUS MARINUS* IN OYSTER TISSUES.

Christopher F. Dungan* and Rosalee M. Hamilton, Cooperative Oxford Laboratory, Oxford, MD 21654.

Enzyme-Linked Immunosorbent Assays (ELISA) represent one of the most sensitive immunoassay formats, and are widely used for clinical disease diagnosis and pathogen detection. In addition to their sensitivity attributes, rapid ELISA assays performed in high-sample capacity 96-well microtiter plates use low sample and reagent volumes, employ labor-saving multichannel micropipettes for reagent delivery and washing, and may be completely automated. We have developed a prototype ELISA assay for detecting *P. marinus* in oyster tissue homogenates, which employed polyclonal rabbit antibodies adsorbed to polystyrene microplate wells to selectively capture and immobilize pathogen antigens from oyster tissue samples, on the assay solid phase. Immobilized *P. marinus* antigens were subsequently detected using biotinylated versions of the same antibodies, whose binding was signaled enzymatically via streptavidin-enzyme conjugates or complexes. This crude ELISA assay detected as little as 1.5 ng of *P. marinus* protein in microplate wells also containing 100 μg of normal oys-

ter protein, with colorimetric assay signals proportional to pathogen protein concentrations. The ELISA assay routinely detected low-intensity *P. marinus* infections in clinical samples, as well as infections not detected by Ray's thioglycollate assays of paired rectal or hemolymph tissue samples.

JUVENILE OYSTER DISEASE RESISTANCE STUDIES: 1994–1996. C. Austin Farley* and E. J. Lewis, National Marine Fisheries Service/NOAA, Cooperative Oxford Laboratory, 904 S. Morris Street, Oxford, MD 21654; David Relyea and Joseph Zahita, Frank M. Flower Co., Oyster Bay, NY 11771; Gregg Rivara, Cornell University Cooperative Extension, Southold, NY 11971.

This is the third year of juvenile oyster disease (JOD) resistance studies in *Crassostrea virginica*. The first year showed up to seven times better survival of progeny of a brood stock selected on the basis of (1) survival and (2) presence of characteristic shell checks. F_1 and F_2 progeny were evaluated the second year against progeny from susceptible brood stocks deployed in seven different sites. Survival of F_1 and F_2 resistant seed was 7 to 25 times better than the susceptible seed.

In 1996, we developed an F_3 generation and compared it with the F_2 progeny (FMF) from Flower Co. brood stocks and a comparable-aged susceptible control population from naive natural Connecticut brood stocks (FCT). Surviving 1995 FCT progeny were used as brood stocks to produce an F_1 resistant FCT strain. Seed were deployed in five different sites in Long Island waters: site 1—Oyster Bay, Long Island Sound FCT- F_1 (not deployed here); site 2—Mattatuck Inlet, Long Island Sound; site 3—Cedar Beach, Peconic Bay; site 4—a tidal pond, Peconic Bay; and site 5—Moriches Bay, Great South Bay. Results after 11 weeks of exposure demonstrated mortalities of 55–84% in the susceptible populations compared with 3–29% for the three resistant populations. No significant differences were seen between the three resistant populations. Survival was 2.5 to 35 times better in the resistant populations.

Management strategy using resistant seed has resulted in increased production to above pre-JOD levels and has eliminated the devastating effects of this disease.

CHANGES IN PROTEASE EXPRESSION BY *PERKINSUS MARINUS* CULTURES FOLLOWING INCUBATION IN RAY'S FLUID THIOGLYCOLLATE MEDIUM. Jerome F. La Peyre,* Office of Sea Grant Development, Louisiana State University, Baton Rouge, 70803; Richard K. Cooper, Department of Veterinary Science, Louisiana State University, Baton Rouge, LA 70803.

Previous studies have shown major differences in protease expression between *Perkinsus marinus* isolates. Protease expression might relate to the initial stage of *P. marinus* used to initiate

cultures. For example, cell-free supernatants of cultures established from infected oyster heart had the highest proteolytic activity and showed major protease bands with approximate molecular weights ranging from 35 to 55 kDa as determined by gelatin-impregnated sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). In contrast, supernatants of cultures established from hyphospores had consistently lower proteolytic activities and showed protease bands with approximate molecular weights of 70–200 kDa.

Changes in protease expression could be produced when cells from cultures originally established from infected oyster heart were incubated in Ray's fluid thioglycollate medium (RFTM). Hyphospores obtained in RFTM readily divided when returned to culture medium and the supernatants of cultures derived from these cells had relatively low proteolytic activity and major protease bands with molecular weights in the 70–200 kDa range. Analysis of supernatant proteins by SDS-PAGE and silver staining, under non-reducing and reducing conditions suggested that all protease bands may represent post-translational modifications of a single gene product. The gene for this protease is being identified to assist in understanding the expression of this putative virulence factor of *P. marinus*.

JUVENILE OYSTER DISEASE EXPERIMENTAL STUDIES: 1995–1996. Earl J. Lewis* and C. Austin Farley, National Marine Fisheries Service/NOAA, Cooperative Oxford Laboratory, 904 S. Morris Street, Oxford, MD 21654; Rocco Cipriano, National Fish Health Research Laboratory, 1700 Leetown Road, Kearneysville, WV 25430; Eugene B. Small, University of Maryland, Department of Zoology, College Park, MD 20742.

Juvenile oyster disease (JOD) is a recent and often fatal malady of young cultured oysters, *Crassostrea virginica*, from areas of the northeastern United States. Bacterial and protistan agents have been investigated as causative agents of JOD, but the cause of the disease remains unknown.

The disease was found to be readily transmissible in laboratory studies using infected oysters, or material filtered from the water column at one affected facility. Attempts were also made to transmit JOD to other cultured oysters, *C. gigas* and *Ostrea edulis*, and to uninfected *C. virginica* from clams reared in the same facility as JOD-infected oysters. Both *C. gigas* and *O. edulis* showed elevated mortalities, but histological examinations have not been completed for comparison to JOD. However, there has been no report of mortalities in *O. edulis* reared near JOD-infected *C. virginica* in the Northeast. No evidence of transmission was found from clams to oysters.

Seventeen species of vibrio and 32 species of other bacteria were isolated from oysters in our bacterial studies. Commonly recovered bacteria were isolated nearly as frequently from JOD-infected as uninfected oysters. Ciliates were routinely isolated from JOD-infected oysters, but rarely from uninfected oysters.

CHOPTANK RIVER OYSTER RECOVERY PROJECT. Don Meritt,* Horn Point Environmental Lab, University of Maryland; Pat Gaffney, College of Marine Studies, University of Delaware; Ken Paynter, Department of Zoology, University of Maryland, College Park, MD 20742.

The Maryland Oyster Roundtable Action Plan called for the construction of experimental oyster bars to test the feasibility of using hatchery reared seed to 'reconstruct' oyster bars which have been over-exploited or ravaged by disease and are currently devoid of oysters. In 1995, 5 acres of a natural oyster bar, built up with dredged shell, was planted with approximately 2.5 million oyster spat produced in the Horn Point Environmental Laboratory oyster hatchery. Using SCUBA, divers from our lab have been surveying and sampling the planted seed to determine rates of growth, mortality and disease acquisition. In addition, we have deployed instruments for continuous monitoring of benthic water quality including dissolved oxygen, pH, temperature and salinity. From this project, we hope to better understand how water quality in the benthic habitat affects disease acquisition and progression in oysters. The project will continue through at least 1997 to determine whether or not hatchery reared seed represents a valuable asset to the oyster industry in the Chesapeake Bay region. Participation in this project will allow us to better understand how environmental conditions in the benthic habitat affect the physiology of the oyster. As these oysters grow, we will begin sampling hemolymph from them to determine how blood chemistries like pH, calcium levels and related factors vary with the changing environment. In addition, as they become infected with *Perkinsus marinus* (which is highly likely) we will continue to monitor blood chemistries to learn more about the physiological affects of the parasite on the oyster.

CELLULAR VOLUME REGULATION IN, PERKINSUS MARINUS, A PROTOZOAN PARASITE OF THE EASTERN OYSTER, CRASSOSTREA VIRGINICA. Kennedy T. Paynter,* Christine Parker, and Amy Beaven, Department of Zoology, University of Maryland, College Park, MD 20742.

Perkinsus marinus, a protozoan parasite of the eastern oyster, *Crassostrea virginica*, apparently has the ability to regulate its cell volume as ambient osmotic pressure changes. Recent studies have shown that *P. marinus* cells swell minutes after an acute osmotic shock but return to near normal volumes soon after the shock. This kind of cell volume regulation is typically accomplished by manipulating intracellular osmolytes. For instance, oyster gill tissues will release large amounts of free amino acids after hypoosmotic shock to reduce intracellular osmotic pressure thus bringing intracellular osmotic pressure more into balance with extracellular osmotic pressures and restoring cell functions. The molecules usually employed as osmotic effectors by protozoans are either free amino acids or polyalcoholic sugars. We have examined

the free amino acid composition of cultured *P. marinus* cells before and after acute osmotic stress.

P. marinus cells acclimated to 35 ppt contain 226 nmol/cell of free amino acids. Cells acclimated to 10 ppt, however, contain only 46 nmol/cell. We propose that *P. marinus* regulates cells volume by manipulating intracellular free amino acid concentrations. Experiments testing this hypothesis are ongoing.

THE POTENTIAL FOR TRANSMISSION OF PERKINSUS MARINUS BY FECAL MATTER FROM THE EASTERN OYSTER, CRASSOSTREA VIRGINICA. Christine H. Scanlon,* Lisa M. Ragone Calvo, and Eugene M. Bureson, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Transmission of *Perkinsus marinus*, an important pathogen of the eastern oyster, *Crassostrea virginica*, has been thought to occur via the dispersal of infective *P. marinus* cells upon death and decomposition of infected oysters. However, recent studies have demonstrated the presence of *P. marinus* in fecal matter from live, heavily infected oysters. It has been hypothesized that fecal elimination of *P. marinus* cells may be an important mechanism for transmission, as well as a nondestructive method for estimating infection intensity. The purpose of this study was to examine the role of fecal matter in direct transmission of the parasite. Three experiments were conducted to elucidate this role. In the first experiment, the abundance of *P. marinus* in the hemolymph and feces of individual oysters was monitored over a period of five months in order to determine the correlation of fecal parasite abundance with infection intensity as estimated from the oyster hemolymph. Preliminary data to date suggest that the abundance of *P. marinus* cells in the feces of infected oysters roughly correlates with levels of *P. marinus* found in the hemolymph. The second and third experiments were conducted to determine if the fecal matter from *P. marinus* infected oysters is infective to previously uninfected oysters. In the second experiment, uninfected oysters were dosed with feces from infected oysters, and in the third experiment, uninfected oysters were paired with infected oysters in individual containers. The results of these infectivity experiments have not yet been determined.

IDENTIFICATION OF SUPEROXIDE DISMUTASE cDNA FROM PERKINSUS MARINUS. Anita C. Wright* and Gerardo R. Vasta, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E. Pratt St., Baltimore, MD 21202.

Perkinsus marinus is a facultative intracellular parasite and pathogen of the Eastern oyster, *Crassostrea virginica*. Previously, the oxidative burst which usually accompanies the phagocytic process in oyster hemocytes has been shown to be absent during the

uptake of *P. marinus*. Superoxide dismutase (SOD), which has been postulated to be a virulence determinant for both prokaryotic and eukaryotic pathogens, is an effective scavenger of lethal reactive oxygen intermediates resulting from phagocytic cell activation. Therefore, we examined *P. marinus* expression of SOD using cDNA characterization. *P. marinus* RNA was reversed transcribed by standard methods, and cDNA amplified by polymerase chain reaction (PCR) using primers derived from conserved regions of previously described protozoan iron SOD. Sequence revealed expression of two distinct SOD RNAs (Pmsod1 and Pmsod2). Complete cDNA sequence for both SODs was obtained by rapid amplification of cDNA ends (RACE) and subsequent fusion of 5' and 3' RACE products. Identity of the deduced amino acid sequence was only 32.6% between Pmsod1 and Pmsod2. Comparison of Pmsod1 to available sequences showed greatest identity to amino acid sequence from *Plasmodium falciparum* (53.9%), while Pmsod2 was more divergent and had greatest identity to *Bordetella pertussis* (43.5%) with slightly less to *P. falciparum* (42.3%). Primary sequence indicated that PmSODs belong to either iron or manganese types (not Cu/Zn) but could not distinguish between these types. These sequences are the first description of superoxide dismutase in *P. marinus* and indicate the expression of a potential virulence factor for this organism.

MOLLUSCAN DISEASE II

BIOCHEMISTRY OF THE PHAGOSOME IN OYSTER HEMOCYTES. Amy Beaven,* Kennedy T. Paynter, and Jennifer Wojcik, Department of Zoology, University of Maryland, College Park, MD 20742.

We have investigated various aspects of phagocytosis and chemiluminescence (CL) in hemocytes from the eastern oyster, *Crassostrea virginica*, and found that inclusion of buffers in the phagocytosis assay dramatically reduces chemiluminescence. HEPES, MOPS and phosphate buffers all reduced CL by over 90% at 20mM concentrations. Furthermore, myeloperoxidase (MPO) activity in extracts of oyster hemocytes was measured using tetramethylbenzidine (TMB). TMB peroxidation had a pH optimum of approximately 5.5 indicating that phagosome acidification may be an important step in cellular killing by oyster blood cells.

Using fluorescent techniques we investigated the conditions within the phagosome of oyster blood cells stimulated to engulf zymosan particles. Experiments using the pH-sensitive fluor DM-NERF conjugated to zymosan granules indicate that the phagosome lumen in oyster cells is acidified soon after formation of the phagosome. This is similar to the mechanism observed in vertebrate macrophage cells where acidification apparently serves to activate lysosomal enzymes. Studies are currently underway to investigate the effect of *Perkinsus marinus* cells on phagosome formation and cellular killing mechanisms in oyster blood cells.

A NEW AND UNUSUAL SPECIES OF PERKINSUS PATHOGENIC TO CULTURED JAPANESE SCALLOPS, PATINOPECTEN YESSOENSIS, IN BRITISH COLUMBIA, CANADA. Susan M. Bower,* Janice Blackburn, and Gary R. Meyer, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, British Columbia, Canada, V9R 5K6.

A new species of *Perkinsus*, commonly called SPX (Scallop Protozoan X), occurred sporadically and caused disease among Japanese scallops during grow-out in British Columbia. Creamy-white pustules in the connective tissue of all organs and up to 60% mortalities occurred in adult scallops. In juveniles less than 5 cm in shell height, tissue lesions were often not apparent but, mortalities caused by SPX approached 100% in some localities. Most developmental stages and the ultrastructural morphology of the zoospore were similar to that of *Perkinsus* spp. However, unlike all other known *Perkinsus*: (1) the thioglycollate culture test, which is diagnostic for *Perkinsus* spp., was negative for SPX, (2) zoospores of SPX have only been observed in the edematous tissues spaces of about 15% of heavily infected and living juvenile scallops (<5 cm shell height) whereas zoospores of other *Perkinsus* do not occur within the tissues of the molluscan host and (3) pathogenic SPX infections with associated high mortalities have developed in scallops at about 9°C while disease attributable to all other *Perkinsus* have been reported from molluscs in the tropics or in temperate waters during hot summer months. The lethal affects of SPX can be mitigated by culturing hybrid scallops: crosses between Japanese scallops that have survived SPX epizootics and weathervane scallops (*Patinopecten caurinus*), a species native to British Columbia.

GENERATION OF REACTIVE OXYGEN SPECIES BY CRASSOSTREA VIRGINICA HEMOCYTES IN RESPONSE TO LISTONELLA ANGUILLARUM. Lisa A. Bramble* and Robert S. Anderson, Chesapeake Biological Laboratory, Center for Environmental and Estuarine Studies, University of Maryland, Solomons, MD 20688.

By analogy to mammalian immune reactions, it has been proposed that reactive oxygen species (ROS) produced by bivalve hemocytes in response to membrane stimulation (e.g., by zymosan) similarly contribute to host internal defense mechanisms. As an initial step in exploring this hypothesis, we assessed the ability of the opportunistic bacterial pathogen, *Listonella* (formerly *Vibrio*) *anguillarum* to stimulate hemocyte-derived luminol- and lucigenin-augmented chemiluminescence (CL). While heat-killed *L. anguillarum* stimulated lucigenin CL (reportedly specific for the superoxide anion), viable bacteria failed to do so. In the luminol CL assay (thought to measure the activity of the hydrogen peroxide-myeloperoxidase-halide system), neither viable nor heat-killed *L. anguillarum* enhanced hemocyte-derived CL. Experimental data indicate that the inability of viable *L. anguillarum* to stimulate phagocyte-generated lucigenin and luminol CL may be attributable

to suppression of ROS by the bacterial antioxidant enzymes superoxide dismutase and catalase, respectively. The results suggest that, under the conditions employed in the CL assays, bactericidal activity towards *L. anguillarum* would not be mediated by ROS.

DIRECT OBSERVATIONS OF FEEDING BEHAVIOR OF THE PARASITIC TURBELLARIAN *URASTOMA CYPRI-NAE* IN OYSTERS *CRASSOSTREA VIRGINICA*. Nicole T. Brun* and Andrew D. Boghen, Biology Department, Université de Moncton, Moncton, NB, Canada E1A 3E9.

To date, research on molluscan diseases has focused primarily on microbial and protozoan pathogens. Less attention has been attributed to the potential impact of metazoan parasites. The Turbellarian *Urastoma cyprinae* has been reported on the gills of various bivalve species in different regions of the world. Contrary to earlier interpretations that *U. cyprinae* is an occasional commensal, recent investigations have demonstrated that the gill-worm can have negative effects on its molluscan host, and, that at least in certain instances is parasitic. Furthermore, our work shows that there is a strong attraction by *U. cyprinae* to oysters, and that this is probably induced by gill tissue. To gain clarification of the host-parasite relationship and acquire basic information on what and how the worm is feeding, a three-part project incorporating morphological, analytical and behavioral studies using endoscopic techniques is in progress. In the latter case, findings reveal that the worms are distributed throughout the gill tissue, but that they are most heavily concentrated along the dorsal ciliary tracts. Stabilizing and probing activities of *U. cyprinae* are associated with morpho-physiological features localized at the anterior end. While the degree of contact between the oral-genital pore situated in the worm's posterior end and host tissue varies, extended and more intimate contact is observed between the worm's body and heavily mucus-coated surfaces. Specific behavioral activities such as body arching and constant repositioning, not only sustain but optimize host-parasite intimacy. This suggests that the tegument plays a major role in food acquisition and transfer—a phenomenon supported by histological and ultrastructural findings.

OCCURRENCE OF QPX, QUAHOG PARASITE UNKNOWN IN VIRGINIA HARD CLAMS, *MERCENARIA MERCENARIA*. Lisa M. Ragone Calvo, Juanita G. Walker, and Eugene M. Bureson,* Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

In July 1996, in response to industry concerns, the Virginia Institute of Marine Science (VIMS) initiated a sampling program to examine wild and cultured hard clams, *Mercenaria mercenaria*, for QPX, Quahog Parasite Unknown, a protozoan parasite that has been associated with severe mortalities of hard clams in localized areas in Canada and New England. Analysis of our initial samples from three sites revealed the presence of the parasite in 1–2 year old cultured clams at a Chincoteague Bay, VA grow-out location.

This is the first report of QPX in Virginia. Prevalence was low, 8%, and infections were localized and low in intensity. There was considerable evidence that the clams were mounting an effective immune response as numerous parasite cells were dead and there was no indication of QPX-associated mortality of hard clams in Chincoteague Bay. In response to the observation of QPX in Virginia clams, VIMS expanded its monthly survey of hard clams for QPX and in August 1996, clams were sampled from the original three sample sites and from ten additional sample sites. An effort was made to survey wild and cultured clams from western and eastern shore areas where clam harvest and grow-out take place. QPX was again present in cultured clams collected at the Chincoteague Bay site. The prevalence of QPX in the August sample was 20%. QPX was also observed at a prevalence of 8% in cultured clams from Sandy Island which is also located on the seashore of Virginia's eastern shore. Infections in clams from both locations were again localized and low in intensity. We will continue to monitor selected sites at 4–8 week intervals and the results of our monitoring efforts will be presented.

DEVELOPMENT OF A RAPID IDENTIFICATION SYSTEM TO STUDY THE NATURAL FLORA OF THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*. Maya A. Crosby,* Katherine J. Boettcher, and Bruce J. Barber, School of Marine Sciences, University of Maine, Orono, ME 04469.

Studies of the natural bacterial flora of shellfish have been hindered by the use of bacteriological identification procedures that were developed for common clinical strains of bacteria. Commercial kits and automated technologies (such as API20E strips and the Biolog system) are expensive and have not shown highly accurate results when used for estuarine bacteria. Further, there is no adequate standardized method for the isolation and identification of shellfish bacteria other than strains of public health concern. Researchers use a variety of techniques, often with conflicting results. This is of great significance in the study of shellfish diseases with a bacterial etiology. Thus, there is clearly a need for a battery of bacteriological tests that would function as a rapid, cost-effective and accurate identification method optimized for use with estuarine bacteria.

For this purpose, we proposed a system combining traditional media-based identification tests and pre-prepared disks, to define the natural flora of *Crassostrea virginica* in the Damariscotta River estuary. Oysters were homogenized and the resulting suspensions were diluted and plated on seawater agar. Isolates were then examined using a variety of identification tests including: staining, incubation on various media, and the application of disks impregnated with amino acids, sugars, and bacteriostatic agents. In the majority of tests a conspicuous color change indicated a positive or negative reaction. Preliminary results showed that the predominant genera were *Pseudomonas*, *Alteromonas*, and *Alcaligenes*, followed by *Vibrio* and *Micrococcus*. We also observed

Cytophaga-Flexibacter, *Flavobacteria*, and *Acinetobacter* in lesser numbers.

SARCOMA IN THE SOFTSHELL CLAM (*MYA ARENARIA*): EFFECTS ON PLASMA PROTEASE INHIBITORS. Ehab Elsayed and Mohamed Faisal,* School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062; Shawn M. McLaughlin, National Marine Fisheries Service, Cooperative Oxford Laboratory, 904 S. Morris St., Oxford, MD 21654.

Disseminated sarcoma causes significant mortalities in soft-shell clam (*Mya arenaria*) populations along the east coast of the United States. Reduced cellular defense mechanisms have been reported in sarcomatous clams; however, the biochemical basis of this decreased activity is not fully understood. In the present study, we provide evidence on the presence of protease inhibitors in the hemolymph of softshell clams. The levels of protease inhibitory activity varied greatly from one enzyme to another. For example, 1 μ g of plasma protein inhibited 595 ± 175 ng of pepsin (aspartic protease), 5 ± 2 ng of papain (cysteine protease) and 3 ± 1 ng of trypsin (serine protease). In addition, softshell clam plasma displayed anti-metalloprotease activity but at significantly lower levels. The effects of sarcoma progression on plasma protease inhibitory activities were investigated. Clams with early and intermediate stages of sarcoma showed a non-significant decrease in the levels of protease inhibitors. However, clams with advanced sarcomas showed a marked decrease in the ability to inhibit trypsin, papain, and pepsin while the protease inhibitory activity levels against metalloprotease were completely exhausted. The levels of inhibition against chymotrypsin (also a serine protease) showed, however, a significant increase. The mechanism leading to this suppression is being investigated.

SUMMER MORTALITY AND THE STRESS RESPONSE OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS* THUNBERG. Carolyn S. Friedman,* California Department of Fish & Game, c/o Bodega Marine Laboratory, Bodega Bay, CA 94923; Ally Shamseldin and Murali Pillai, Sonoma State University, Rohnert Park, CA 94928; Paul G. Olin, California Sea Grant Extension, Santa Rosa, CA 95403; Gary N. Cherr, Susan A. Jackson, Erik Rilkin, K. R. Uhlinger, and James S. Clegg, University of California, Bodega Marine Laboratory, Bodega Bay, CA 94923.

Summer mortalities of *Crassostrea gigas* in Tomales Bay, California have approached 52% in 1993 and 63% in 1994. It is likely that multiple chronic stresses may combine to bring about mortalities. In order to examine possible etiologies of *C. gigas* losses in Tomales Bay, we monitored several environmental parameters in conjunction with a sentinel study in which seed from two sources were planted at 3 farms in the bay. Mortality was observed in late July and August of 1995 and was associated with water

temperatures above 20°C and a bloom of *Gymnodinium splendens*. Microscopic examination of stained tissue sections during a mortality episode revealed a diffuse to acute and multi-focal inflammation surrounding digestive tubules, thickening of peritubular muscularis and connective tissue, and increased vacuolization and dilation of digestive tubules. These data suggest that mortalities are related to environmental causes and not an infectious agent. Thermotolerance was induced in Pacific oysters by heat shock (HS) which enabled them to withstand an otherwise lethal temperature. Thermotolerance was maintained for up to two weeks following HS and was accompanied by the appearance of an inducible isoform of the stress protein (SP)-70 family via synthesis. This induced SP has a molecular weight of 69 kDa and is observed in gill, mantle, and heart tissues and may be a useful biomarker for stress in Pacific oysters.

ISOLATION OF *PERKINSUS* SP. FROM THE SOFTSHELL CLAM (*MYA ARENARIA*). Shawn M. McLaughlin, National Marine Fisheries Service, Cooperative Oxford Laboratory, 904 S. Morris St., Oxford, MD, 21654; Mohamed Faisal,* School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

A noticeable increase in prevalences of *Perkinsus* sp. has occurred since the late 1980's in softshell clam populations surveyed in the upper Chesapeake Bay. Histological analyses typically show encapsulation and degeneration of *Perkinsus* sp. in host tissues; however, advanced infections may become systemic. In this study, *Perkinsus* sp. was isolated from the hemolymph of a softshell clam (*Mya arenaria*) and cultured *in vitro*. The cultured organism was isolated from the hemolymph of a softshell clam collected from Swan Point, Chester River, Maryland. *Perkinsus* sp. cells were cloned in their third week of subculture. Gill tissue from this clam developed hyphospores in Ray's Fluid Thioglycolate Medium (RFTM) which stained blue-black with Lugol's solution. The cultured protozoan grows well at 20 and 28°C in the presence or absence of CO₂ tension using a chemically defined, protein free medium. The clam *Perkinsus* sp. isolate shares morphological similarities with the cultured *Perkinsus marinus* isolate. Perkinsus-1, originally isolated from the oyster, *Crassostrea virginica*. Analysis of extracellular proteins by gelatin-impregnated gel electrophoresis indicates the softshell clam *Perkinsus* sp. isolate was similar, but not identical, to the patterns formed by Perkinsus-1.

QUAHOG PARASITE UNKNOWN (QPX): AN EMERGING DISEASE OF HARD CLAMS. Roxanna Smolowitz,* LAAMP, University of Pennsylvania, MBL, Woods Hole, MA 02543; Dale Leavitt, Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, MA 02543.

During the summer of 1995, Smolowitz and Leavitt investigated a cause of annually, increasing severe morbidity and mortality in cultured sublegally-sized hard clams being experienced by

several hard clam aquaculturists in Provincetown, MA. QPX, a protistan parasite, was identified in the small sample of animals examined. However, a significant bacterial infection was also present in most clams of this sample. With additional funding, Smolowitz and Leavitt were able to collect a large sample of animals from two severely affected leases in October, 1995 for examination. QPX was identified 90% of the animals selected as being affected based on gross signs of poor growth, slight gapping and chips on the shell edges. In November, 1995, QPX was again identified in hard clams which had experienced an acute episode of severe mortality from a clam lease in Duxbury, MA.

Subsequently, QPX has been identified in lease planted, sub-legal-sized hard clams in Provincetown and Duxbury clams leases that originating from several hatchery/nurseries. The infection is first detected in the clams approximately one year after planting in the leases. Seed from four nurseries that supply the cape aquaculturists were examined as well as adults and subadults from several other areas on Cape Cod.

At gross necropsy of affected hard clams from the Provincetown and Duxbury leases, diffusely swollen mantle edges or distinct nodules are noted. Microscopically, foci of parasitic infection are associated with severe granulomatous inflammation, attempted encapsulation and rare multinucleated giant host cells.

LIFE CYCLE STUDIES OF *HAPLOSPORIDIUM NELSONI* (MSX) USING PCR TECHNOLOGY. Nancy A. Stokes,* Brenda Sandy Flores, and Eugene M. Bureson, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062; Kathy A. Alcox, Ximing Guo, and Susan E. Ford, Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08349.

The oyster pathogen *Haplosporidium nelsoni*, the agent of MSX disease, has caused extensive oyster mortality in the eastern United States since 1957. Much has been learned in the past four decades; however, the complete life cycle of *H. nelsoni* remains unknown. Attempts to infect oysters directly with *H. nelsoni* spores have been unsuccessful, thus leading to speculation that parasite transmission between oysters occurs via an obligate intermediate host.

We have developed a diagnostic assay using the polymerase chain reaction (PCR) which detects *H. nelsoni*-infected oysters with much greater sensitivity than traditional histological examination. This assay has been optimized for use with environmental samples and the *H. nelsoni*-specific PCR primers are being used in the search for the putative intermediate host(s). Weekly samples of water and sediment fractions and of macroinvertebrates have been taken from MSX-endemic areas of Delaware Bay and York River, VA since March 1996. Total genomic DNA has been extracted from each sample and subjected to PCR amplification. Some of the samples have yielded *H. nelsoni* PCR product and we are currently optimizing the protocols to conduct in situ hybridizations on these samples using the *H. nelsoni*-specific DNA probe.

OYSTER MANAGEMENT

INTERTIDAL OYSTER REEF HABITAT USE AND FUNCTION: WHAT HAVE WE LEARNED AFTER TWO YEARS? Loren D. Coen,* Elizabeth L. Wenner, David M. Knott, M. Yvonne Bobo, Nancy H. Hadley, Donnia L. Richardson, and Bruce Stender, Marine Resources Research Institute, SCDNR, Charleston, SC, 29412; Rachel Giotta, University of Charleston, Grice Marine Laboratory.

In 1994, we initiated a long-term Oyster Habitat Study (OHS) to examine the importance of intertidal oyster reefs in southeastern estuarine ecosystems. We utilized an experimental approach, constructing three replicate experimental reefs (each ~24 m²) at each of two sites, paired with equivalent-sized natural reefs to better understand habitat development and functioning. One site is "undeveloped" and open to harvesting; the other is a "developed" area adjacent to a marina and closed to harvesting. We have now completed two years of sampling and a preliminary analysis of the transient and resident reef fauna collected and enumerated since 1995. We have also begun to analyze an extensive long-term environmental and oyster life history dataset (e.g., recruitment, disease onset in SPF-oysters, mortality and growth, monthly intensity and prevalence of Dermo and MSX) on the experimental, as well as adjacent natural reefs. For resident sampling, within-site species richness appears to be similar between experimental and natural reefs six months post-construction. Faunal biomass values showed a different pattern, with large numbers of mussels (e.g., *Geukensia*) on natural reefs contributing significantly to biomass differences observed through year 1; significant between-site mussel biomass differences were also observed. Faunal densities were also similar between the two reef types at the developed site; however, the natural reefs at the undeveloped site supported greater resident densities. These findings are discussed as they relate to previous studies using mussels as "sentinels" of environmental quality. For the transient community, no significant differences were detected between experimental and natural reef areas for either overall abundance or species richness, averaged over the three replicate reefs per site. By initiating and following the long-term reef development, we will be able to explore potential changes in reef habitat status and function during reef succession.

THE IMPACT OF STRUCTURAL PARAMETERS ON PRICE SPREAD IN THE OYSTER PROCESSING SECTOR IN THE GULF OF MEXICO. Assane Diagne* and Walter R. Keithly, Jr., Coastal Fisheries Institute, Wetland Resources Building, Louisiana State University, Baton Rouge, LA 70803–7503.

The oyster fishery has been established in the Gulf of Mexico for more than a century and is currently one of the most significant components of the region's seafood industry. About 18 million pounds of oysters, valued at approximately \$33 millions, were landed in the Gulf of Mexico in 1995 (National Marine Fisheries

Statistics, 1996). Along with other seafood species harvested, this sizeable supply of fresh oysters supports a dynamic seafood processing industry that has developed in the region and throughout the Southeastern United States. Following a brief overview of historical landings, this paper evaluates the structure of the oyster processing industry in the Gulf and explains the variations observed in the difference between output and input prices. The structural parameters considered include the number and size distribution of processing firms and their degree of diversification. In addition, the industry's concentration and stability are assessed. Changes in the difference between processed and raw oyster prices will be explained using the structural parameters presented.

RELAYING OF OYSTERS BY LOUISIANA FISHERMEN IN RELATION TO ECONOMIC AND ENVIRONMENTAL FACTORS. Walter R. Keithly, Jr.* and Assane Diagne, Louisiana State University, Coastal Fisheries, Wetlands Resources Building, Baton Rouge, LA 70803–7503; Ronald Dugas, Louisiana Department of Wildlife and Fisheries, 1600 Canal St., New Orleans, LA 70112.

Relaying of oysters from closed to approved waters is a costly process. As such, relaying activities are likely to be more prevalent under certain economic and environmental conditions. One primary factor hypothesized to influence the demand for relaying activities is the output price of the harvested product. As such, as the output price increases, demand for relaying is expected to increase, *ceteris paribus*, due to higher levels of profitability associated with the process. Similarly, scarcity of input, i.e., oyster supply, in approved waters is expected to influence the demand for relaying activities. Specifically, as the relative scarcity of input increases, the demand for relaying activities is hypothesized to increase accordingly, *ceteris paribus*.

The purpose of this paper is to examine oyster relaying activities in Louisiana from 1980 to present and to analyze changes in these activities in relation to changes in economic and environmental factors. Permits issued by the Department of Health and Human Resources, Seafood Sanitation Unit are used as a proxy for the demand for relaying activities. Changes in the number of permits issued over time are related to prices and environmental factors via econometric modelling efforts. Output prices are found to be a highly significant factor in determining the demand for permits (i.e., demand for relaying activities).

NATIVE OYSTER RESTORATIONS IN OREGON. Anja Robinson,* Fisheries & Wildlife, Hatfield Marine Science Center, Oregon State University, Newport, OR 97365; John Johnson, Oregon Department of Fish & Wildlife, Hatfield Marine Science Center, Newport, OR 97365.

The native oyster *Ostrea conchaphila* is the only oyster species native to the west coast of the United States. It was abundant in the bays and estuaries from Alaska to California. Coastal Indians consumed oysters that they could reach during low tides. White set-

tlers began to harvest native oysters in the mid-1800s, and at the end of the century their numbers had decreased to levels too low for commercial harvesting. Added stress from silt loads after logging and pollution from mill operations caused near extinction of native oysters.

The west coast oyster industry depends on the introduced Pacific oyster *Crassostrea gigas*. Seed for the Pacific oyster is produced in hatcheries, since the water temperature of most estuaries is too cold for natural reproduction. Remnants of native oysters reproduce and set on Pacific oysters just to be discarded as commercial oysters are shucked. Rather than waiting for native oyster to inch their way back, action has been taken to reproduce them in a hatchery and plant them in Oregon bays and estuaries. The initial plantings were performed in 1994, and yearly since then. Experimental plantings of native oysters are thriving and more extensive plantings are under way.

A GIS BASED DECISION SUPPORT SYSTEM FOR OYSTER MANAGEMENT IN MOBILE BAY, ALABAMA. Leonard J. Rodgers* and David B. Rouse, Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL 36849.

The American oyster (*Crassostrea virginica*) has been a major component of the Alabama seafood industry for more than a century. The resource has been limited in extent, with only about 2000 acres of very productive reefs and 1000 acres of marginally productive reefs. Production has historically been cyclical, but comparatively stable. Fluctuations in harvest are influenced by fresh water inflow, recruitment, habitat availability, predator abundance, disease, water quality, and human harvest, to name a few.

To support interest in expansion of the oyster industry a decision support system has been developed using GIS tools which simulates the effects of management and culture practices on the oyster resources of Mobile Bay. The system incorporates the spatial as well as the qualitative effects of management alternatives, so that the manager will be better able to predict where and which alternative management practices should or should not be attempted.

PARTICLE PROCESSING IN BIVALVES: CAPTURE, TRANSPORT, SELECTION

THE ROLE OF MUCUS IN PARTICLE PROCESSING BY SUSPENSION-FEEDING MARINE BIVALVES: UNIFYING PRINCIPLES FROM DIVERSE SYSTEMS. Peter G. Beninger,* Département de Biologie, Université de Moncton, Moncton N.B. Canada E1A 3E9; Harold Silverman, John W. Lynn, and Thomas Dietz, Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA.

Contemporary research on bivalve suspension-feeding mechanisms has revealed a diversity of particle processing depending on

the anatomy and functioning of the pallial organs involved. On the biochemical level, however, some evidence of homogeneity has emerged concerning the role of mucus in these processes. The present study explores this theme using laser confocal microscopy, video endoscopy and mucocyte mapping. Five species representing five different families and all four major gill types are represented: *Mytilus edulis*, *Placopecten magellanicus*, *Crassostrea virginica*, *Mya arenaria*, and *Spisula solidissima*. Confocal laser investigations on *M. edulis* and *C. virginica* provide the first direct confirmation of the two-layer model of mucociliary transport in any organism. Viscous acid (AMPS) or acid-dominant mucopolysaccharides (ADMPS) are used when particle transport occurs on an exposed surface, or on a structure leading directly to such a surface, counter to the prevailing current flow. Associated functions are indiscriminate transport in gill ventral particle grooves and rejection of pseudofeces. Lower-viscosity mixed mucopolysaccharides (MMPS) are used when particle transport is on an enclosed or semi-enclosed surface, leading to other such surfaces, and with the current flow. Associated functions are transport of particles destined for ingestion, and ingestion itself. Low-viscosity neutral mucopolysaccharides (NMPS) are found in regions where reduction of mucus viscosity is important, such as the areas of the labial palps responsible for fluidization of the high-viscosity mucus-particle cord of the gill ventral particle groove prior to particle extraction. There thus appears to be a specialization of mucus type corresponding to functional specialization of the various pallial organs in suspension-feeding marine bivalves.

PARTICLE PROCESSING MECHANISMS OF THE EULAMELLIBRANCH BIVALVES *SPISULA SOLIDISSIMA* AND *MYA ARENARIA*. Suzanne C. Dufour,* Peter G. Benninger, and Julie Bourque, Département de Biologie, Université de Moncton, Moncton N.B. Canada E1A 3E9.

Particle processing in eulamellibranchs is the least well-known of the four principal marine suspension-feeding bivalve gill types. Particle treatment on the pallial organs (gills, palps, lips, mantle) of *Spisula solidissima* and *Mya arenaria* was examined using endoscopy and histology, as well as half-shell preparations. In both species, all particles intercepted by the gill were transported ventrally to the gill particle groove and then anteriorly to the labial palps. Rejected particles (i.e. pseudofeces) were shunted to the palp ventral margin, and thence posteriorly to the palp tip and ultimately the mantle. Pseudofeces were transported along a narrow, distinct pathway on the ventral margin of the mantle to the inhalent siphon. The transport medium for particles on the gill was acid mucopolysaccharides (AMPS). Examination of mucocyte distribution and residual AMPS suggests that in *Mya arenaria*, and perhaps also *Spisula solidissima*, AMPS is secreted onto the gill filament frontal surface from cells remotely located on the lateral faces of the filament. In *Mya arenaria*, mucus-particle masses destined for ingestion were mechanically fluidized by the labial

palps. The presence of neutral mucopolysaccharide (NMPS)—containing mucocytes in the gill particle groove suggests that there may also be a biochemical component to fluidization. Ingestion volume control was effected in both species at two levels: closure of the gill particle groove, and closure of the lower lip of the mouth. Although few differences in pseudofeces pathways were observed between specimens examined endoscopically and in half-shell preparations, the latter were not suitable for study of particle processing for ingestion.

VIDEOMICROSCOPIC STUDIES OF SUSPENSION FEEDING: IT'S A SMALL WORLD. Michael W. Hart, Section of Evolution and Ecology, University of California, Davis, CA 95616.

Suspension feeders depend on some familiar and some nonintuitive mechanisms for concentrating small particles from low concentrations in seawater. Most of these mechanisms operate under conditions characterized by low Reynolds numbers (short distances, small structures, low speeds), where the viscosity of water dominates interactions between food particles and capturing structures. These mechanisms constrain the range and rate of captured particles, thus affecting processes like growth and maturation that depend on food intake. Early studies frequently confused concentration of particles with postcapture transport, packaging, sorting, and ingestion. However, data from direct observations of particle capture using videomicroscopy have shed some light on how various aquatic invertebrates capture food. Until very recently, small suspension feeders (such as the larvae of echinoderms and gastropods, or the single polyps of bryozoans) have proved easier to study using videomicroscopy than have large suspension feeders (such as bivalves). I will review some recent advances on both fronts, discuss the possible reasons for the difficulty in studying large suspension feeders, and suggest some useful recipes for deciphering suspension feeding mechanisms using data from videomicroscopic observations.

MUSCULAR REGULATION OF INTERFILAMENT DISTANCE AND OSTIAL DIMENSION IN THREE SPECIES OF FRESHWATER BIVALVES. Scott Medler* and Harold Silverman, Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

Interfilament and ostial dimensions are important factors related to water flow across bivalve gills. Models for water movement often use a fixed estimate of these passages to estimate pump rate and interfilament water flow velocity. Yet, there is evidence that these flow passageways can change in shape and area over time in living gills. The organization of gill connective tissue and intrinsic musculature suggests that muscular control of interfilament distance and ostial area is possible in the three species examined here. *Toxolasma texasensis* (a freshwater unionid), *Corbicula fluminea*, and *Dreissena polymorpha* all have an intrinsic

muscular system antagonized by a connective tissue skeleton. Specific musculature is organized such that contraction causes a change in interfilament distance and ostial area. The muscle-connective tissue arrangement in all three species is fundamentally similar. Video recordings of living excised gills reveal dynamic alteration of ostial size and shape in response to acetylcholine, FMRFamide, and serotonin. For *D. polymorpha*, exogenous acetylcholine and FMRFamide have excitatory effects while serotonin relaxes the musculature. These studies indirectly suggest a role for muscular regulation of water flow through the bivalve gill.

THE EFFECTS OF CURRENT SPEED ON EXHALENT SIPHON AREA AND SHELL GAPE IN BLUE MUSSELS UNDER CONSTANT SESTON REGIMES. Carter R. Newell,* Great Eastern Mussel Farms, Inc., P.O. Box 141, Tenants Harbor, ME 04860; David J. Wildish, Department of Fisheries and Oceans, St. Andrews, New Brunswick, Canada E0G 2X0.

Investigations concerning the coupling of seston flux with consumption by mussels at bottom lease sites in Maine have been hampered by a lack of understanding of the effects of current *per se*, independent of food particle concentration. A study was performed at the recirculating flume at St. Andrews biological station in which individual blue mussels, *Mytilus edulis*, were affixed to a stand normal to current direction. Distances between the valves (shell gape) and exhalant siphon areas were measured using a time-lapse video apparatus and image analysis software. Animals were fed a ration of two species of cultured algae and mudflat sediment, and current speed was varied from 5 to 30 cm per second while seston remained constant. Siphon area was inversely proportional to current speed, and the shell gape response was reduced at high currents. These results are discussed with respect to the use of the shell gape response as an indirect measurement of pumping rates in filter-feeding bivalves.

SMALL PARTICLE INTERACTION WITH GILL CIRRI IN *CRASSOSTREA VIRGINICA*, *MYTILUS EDULIS* AND *DRESSISSENA POLYMORPHA*: USE OF LASER-CONFOCAL MICROSCOPY FOR HIGH RESOLUTION OBSERVATION OF LIVING TISSUE. Harold Silverman,* John W. Lynn, and Thomas H. Dietz, Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803; Peter G. Beninger, Département de Biologie, Faculté des Sciences, Université de Moncton, Moncton, New Brunswick Canada E1A 3E9.

Many bivalve species can effectively retain particles in the 1 μm size range and individual cilia dimensions on a filament are roughly 0.2 μm in diameter. To allow direct observation of single or a few cilia interacting (or not interacting) with a small particle confocal laser microscopy was used. This technique allows direct observation of individual cilia and in particular larger cirri motion as they interact with small particles. Each of the three species we

observed demonstrated particle interaction with cirri. Particle interaction is defined as cirri and particles approaching to within tenths of micrometers. Direct particle interaction and/or low-Reynolds number paddle type interaction at this scale would be similar in outcome and result directly from a cirral movement. Thus, a freshwater eulamellibranch, a marine filibranch, and a pseudolamellibranch gill all showed close interaction of cirri with 0.7 μm fluorescent latex particles. Particles were moved by the beat of cirri onto the frontal surface of the filament. These observations indicate that individual cirri are involved in interaction with small particles in three of the four main bivalve gill types.

POST-INGESTIVE SELECTION IN LAMELLIBRANCH BIVALVES. Martha G. Smith* and Bruce A. MacDonald, Centre for Coastal Studies and Aquaculture, University of New Brunswick, Saint John, NB, Canada E2L 4L5.

Lamellibranch bivalves have the ability to sort particles within the gut; particles are either passed directly onto the intestine producing poorly digested intestinal feces or are diverted into the digestive gland for further digestion, producing well-digested glandular feces. However, the particle characteristics upon which this selection is based have not been well established. The objective of this first stage of research was to investigate the ability of *Placopecten magellanicus*, *Mytilus edulis* and *Mya arenaria* to sort particles in the gut on the basis of particle size alone. The animals were fed a mixture of microalgae and three sizes (5 μm , 10 μm and 20 μm) of polystyrene beads. Faeces were then collected at intervals, treated with acid and analyzed on a Coulter Multisizer for the presence of beads. Average gut retention times were determined for each bead size and used as an indication of postingestive selection. *P. magellanicus* exhibited postingestive selection in two of five experiments, preferentially retaining larger beads. *M. edulis* and *M. arenaria* showed no evidence of selection in a single experiment. *P. magellanicus* is capable of sorting particles within the gut on the basis of size alone, however, we have yet to determine the role of other particle characteristics (*i.e.* density or chemical properties), in postingestive selection.

FEEDING RESPONSES OF THE EASTERN OYSTER EXPOSED TO VARIOUS CONCENTRATIONS OF SUSPENDED PEAT PARTICLES. K. B. Strychar* and B. A. MacDonald, Centre for Coastal Studies and Aquaculture, University of New Brunswick, Saint John, N.B., Canada E2K 4L5.

Peat mining operations concentrated in the northeastern region of New Brunswick are worth an estimated 43 million dollars per year to the local economy. Concern exists within the shellfish aquaculture industry that particles released by peat mining may negatively influence feeding, growth, and survival of local populations of the Eastern oyster (*Crassostrea virginica*, Gmelin). The objectives of the study were to determine if variable concentrations

of peat particles (2, 5, 10, and 20 mg L⁻¹) suspended in water influence rates of clearance, ingestion, and the efficiency of absorption. Feeding activity of individual oysters exposed to various concentrations of natural and peat particles were assessed using a flow-through feeding apparatus, a peristaltic pump to deliver peat, and a Coulter Multisizer to estimate clearance rates. Clearance rates declined with increasing peat concentrations resulting in relatively constant ingestion rates until a significant increase was observed at 20 mg L⁻¹. Absorption efficiency decreased with increasing concentration until peat particles diluted the background seston resulting in no net absorption at concentrations >5 mg L⁻¹ of peat. If concentrations of suspended peat particles significantly exceed the amount of natural seston they could interfere with energy gain because they are readily ingested but not efficiently absorbed.

MODELING THE DYNAMICS OF PARTICLE PROCESSING IN BIVALVES. J. Evan Ward,* Department of Biological Sciences, Salisbury State University, Salisbury, MD 21801; Jeffrey S. Levinton, Department of Ecology & Evolution, S.U.N.Y., Stony Brook, NY 11794; Sandra E. Shumway, Natural Science Division, Southampton College, Southampton, NY 11968; Terri L. Cucci, Bigelow Laboratory for Ocean Sciences, Boothbay Harbor, ME 04575.

Bivalves are exposed to a particle food supply that fluctuates in quantity and quality along both spatial and temporal scales. The ability to compensate for changes in the particle regime, including adjustments in ingestion rates and rejection of non-nutritive particles as pseudofeces, is critical to the survival of the individual. Due to technical limitations, however, previous models of particle processing have considered only the input (capture/collection) and output (pseudofeces/ingestion) of the system. In contrast, evidence from *in vivo*, endoscopic studies suggest that bivalves are able to make significant adjustments at a much finer scale.

In order to examine fine scale adjustments of the particle-feeding organs, two conceptual models were adopted in this study, both of which treat bivalves as an integrated system of feeding-organ compartments (e.g., ctenidium, palp, gut). In the first (the compartment model), bivalves are modelled as containing a series of particle-processing structures, with characteristic residence times on the structures and transfer points between them. In the second model (the pathway model), particle processing is treated as being analogous to enzyme control systems, with feedback loops that involve interactions between feeding structures that engage in no direct transfer. The validity of the two models were tested using data obtained from endoscopic observations of particle processing in living bivalves. By using the above conceptual approach, we hope to develop a better understanding of the critical limiting factors that mediate particle-feeding in bivalves and the factors that ultimately affect the trophic dynamics of benthic ecosystems.

HYDRODYNAMICS OF PARTICLE CAPTURE IN SUSPENSION-FEEDING BIVALVES: A NEW THEORY BASED ON *IN VIVO* OBSERVATIONS. J. Evan Ward,* Department of Biological Sciences, Salisbury State University, Salisbury, MD 21801; Larry P. Sanford and Roger I.E. Newell, Horn Point Environmental Laboratory, CEES, University of Maryland System, Cambridge, MD 21613; Bruce A. MacDonald, Biology Department, University of New Brunswick, Saint John, NB, Canada E2L 4L5.

The mechanisms by which particles are captured by the ctenidia of bivalves remains poorly defined mainly due to problems associated with studying small-scale processes in animals that are enclosed within an opaque shell. In order to elucidate the capture process, we examined the ctenidia of several species of suspension-feeding bivalves by means of video endoscopy. We found that current theories of particle capture, based on data from isolated ctenidia, do not adequately explain our *in vivo*, endoscopic observations of the capture phenomenon. Using data obtained by our technique, we have developed a new theory of particle capture that focuses on the ctenidial filament as the capture unit. We propose that particle capture is accomplished by direct interception with the ctenidial filament and subsequent mucociliary transport along the frontal surface of the filament. Two primary mechanisms aid in the capture process. First, low angles of approach observed *in vivo* reduce flow drag and increase the efficiency of encounter with the frontal cilia. Second, vortical flow patterns set up by the beating of the laterofrontal cilia or cirri reduce or block flow through the interfilamentar space. Flow is redirected towards the frontal surfaces and through the base of the laterofrontal tracts. This further increases encounter efficiency and promotes particle retention on the frontal surface, at the expense of slightly increased drag. Our study indicates that the suspension-feeding complex as a whole, functions in a manner that is more than merely the sum of its parts, and precludes meaningful results being obtained from surgically altered specimens.

PERKINSUS GENETICS

ELECTROPHORETIC KARYOTYPE OF *PERKINSUS MARINUS* AND KARYOTYPIC DIVERSITY OF *PERKINSUS* SPP. BASED ON ALTERNATING FIELD GEL ELECTROPHORESIS. Thomas J. Burkett* and Gerardo R. Vasta, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 East Pratt St. Baltimore, MD 21202.

The development of techniques for separating large linear DNA molecules by alternating electrical field electrophoresis provides a means for characterizing chromosomes from organisms which have been intractable to genetic or cytogenetic analysis. The analy-

sis of such electrophoretic karyotypes provides novel information on chromosome structure and function. In addition, electrophoretic karyotypes may provide important new information on inter- and intra-species genetic diversity.

We have utilized Contour Clamped Homogenous Electric Field (CHEF) electrophoresis to characterize the size and number of linear chromosomes in *Perkinsus marinus*, as well as other *Perkinsus* spp. We will present the results of this analysis, including an electrophoretic karyotype of *P. marinus* with estimates of total DNA content and ploidy values, as well as a comparison of electrophoretic karyotypes among various *Perkinsus* spp. The results of this analysis will be discussed with respect to species specificity in the host-parasite relationship, as well as the evolutionary position of *Perkinsus* spp.

GENETIC MANIPULATION OF *PERKINSUS MARINUS*: DEVELOPMENT OF INSERTIONAL MUTAGENESIS SYSTEMS AND TRANSFORMATION METHODOLOGIES.

Thomas J. Burkett* and Gerardo R. Vasta, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 East Pratt St. Baltimore, MD 21202.

The ability to propagate *Perkinsus marinus* in axenic culture conditions allows one to investigate the genetic and molecular basis of intracellular parasitism. Unfortunately, genetic studies in *P. marinus* are difficult due to the lack of a sexual cycle. We are attempting to overcome this drawback through the development of molecular genetic approaches for mutagenesis, the identification of mutated genes, and the elucidation of their biological function.

Insertional mutagenesis is a technique for inactivating and marking genes for later cloning and analysis. Based on endogenous transposable elements we are developing an insertional mutagenesis system for use in *Perkinsus* spp. In addition, we are developing methodologies for the incorporation of foreign DNA into *P. marinus* as well as reporter vectors for the characterization of genetic regulatory regions.

We will present data on the identification and characterization of transposable elements from *P. marinus* as well as the development of reporter vectors and transformation technologies. The results of our studies will be presented in the context of developing molecular and genetic tools for the study of intracellular parasitism in *P. marinus*.

PCR DETECTION AND QUANTITATION OF *PERKINSUS MARINUS* IN CHESAPEAKE BAY INVERTEBRATES.

Cathleen A. Coss,* Anita C. Wright, José Antonio F. Robledo, and Gerardo R. Vasta, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD 21202; Gregory M. Ruiz, Smithsonian Environmental Research Center, Edgewater, MD.

Perkinsus-like organisms have been detected in a number of Chesapeake Bay bivalve species other than *Crassostrea virginica*

using the fluid thyoglycollate media assay (FTM). *Perkinsus marinus* may be present in non-oyster invertebrates which could constitute alternative hosts, reservoirs or vectors of this pathogen. The polymerase chain reaction (PCR) is a powerful technique for amplifying specific regions of DNA and has been applied to specific detection of pathogens relevant in aquaculture. The *P. marinus* PCR diagnostic assay specifically amplifies a 307 bp fragment of the non-transcribed spacer region between the 5S and 17S rRNA genes, and two sequence types (type I and type II) differing by six bases have been found for this region. Quantitative PCR methods have also been developed in our laboratory. Bivalves and other invertebrates were collected from oyster beds and/or mud sediments of the James River (VA), Severn River (MD) and Rhode River (MD). Samples which were positive by the PCR diagnostic assay were sequenced directly to determine *P. marinus* type (I or II). Positive PCR results for *P. marinus* have been obtained for *C. virginica* and *Ischadium* sp. from the James River and *Macoma balthica* and *M. mitchelli* from the Rhode River. To date, all sequences match the sequence for *P. marinus* type II. In addition, we are examining the internal spacer regions (ITS 1 and ITS 2) of the rRNA genes and conserved gene sequences such as actin for the *Perkinsus* isolates. Bivalves will continue to be monitored seasonally from the James, Rhode, and Severn Rivers, and *P. marinus* infection density will be quantified by competitive PCR.

ISOLATION OF PROTEIN PHOSPHATASE cDNA FROM *PERKINSUS MARINUS*. Cathleen A. Coss,* Anita C. Wright, and Gerardo R. Vasta, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E. Pratt St., Baltimore, MD 21202.

Protein phosphatases are extremely conserved enzymes which are involved in a variety of regulatory functions essential for cell signal transduction and proliferation. They have been cloned and characterized in a number of protozoans, and in *Leishmania* are protein phosphatases are distinct from the secreted acid phosphatases that have been shown to suppress the oxidative burst of phagocytic cells. In order to understand events that may trigger proliferation of the oyster pathogen, *Perkinsus marinus*, as well as assess pathways that may be relevant to signaling and cell cycle regulation, we attempted to clone and characterize cDNA for protein phosphatase from this organism. RNA was extracted and reverse-transcribed to cDNA according to standard methods. *P. marinus* cDNA was amplified by polymerase chain reaction (PCR) with primers derived from conserved regions of the protein phosphatase 1 (PP1) gene of *Trypanosoma brucei* and based on codon usage analysis from previously sequenced *P. marinus* cDNA (actin and superoxide dismutase). *P. marinus* sequence obtained from PCR products or from clones of PCR products was used to design primers for rapid amplification of cDNA ends (RACE). PCR fu-

sion of 3' and 5' RACE products yielded full length cDNA. *P. marinus* protein phosphatase cDNA showed considerable identity to variety of PPI genes, including *Homo sapiens*, *Schizosaccharomyces pombe*, *Gonyaulax polyedra*, and *Trypanosoma brucei* for both amino acid (71–83%) and DNA (64–72%) sequences. Studies are on-going to characterize expression of this gene and gene product and determine its role in signal transduction for *P. marinus*.

FURTHER STUDIES OF CONSERVED GENES FROM PERKINSUS ISOLATES. José Antonio F. Robledo,* Anita C. Wright, Cathleen A. Coss, and Gerardo R. Vasta, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E. Pratt St., Baltimore, MD 21202; C. L. Goggin, Center for Research on Introduced Marine Pests, CSIRO Division of Fisheries, GPO Box 1538, Hobart 7001 Tasmania, Australia.

Ribosomal RNA genes (rDNA) have been used to distinguish between species of parasites. Other conserved genes such as actin have also been used for phylogenetic studies. Previously, analysis of internal transcribed spacers (ITS1 and ITS2) from the rDNA have shown European isolates of *Perkinsus atlanticus* and Australian isolates of *P. olseni* to be much more closely related to each other than to *P. marinus*. Using primers derived from the non-transcribed space (NTS) domain of rDNA of *P. marinus*, we have shown this region to be specific for this species by polymerase chain reaction (PCR). Moreover, the NTS primers did not amplify *P. atlanticus* or *P. olseni* strains. Actin primers derived from *P. marinus* sequence amplified all three species. Sequences from actin, ITS and NTS regions were obtained from either PCR clones or directly determined from PCR amplification products. NTS sequence from *P. marinus* isolates showed that there are two distinct sequences (Type I and Type II) which differ in six positions for a 307 bp amplified fragment. No differences were found within ITS1 and ITS2 between *P. marinus* types I and II. Additionally, sequence comparison of conserved regions of actin DNA showed *P. marinus* with closer identity to *P. atlanticus* (94.3%) than *P. olseni* (86.8%) strains. It is not clear if ITS of rRNA genes are sufficient for species determination; however, the NTS region could be used as a marker to distinguish between *Perkinsus* strains. Comparison of more genes are needed to answer this question. We are currently looking at rRNA, actin genes, as well as conserved gene sequences from *Perkinsus* isolates from different hosts.

COMPETITIVE PCR FOR QUANTITATIVE ANALYSIS OF PERKINSUS MARINUS. Anita C. Wright, José Antonio F. Robledo, Julie D. Gauthier,* and Gerardo R. Vasta, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E. Pratt St., Baltimore, MD 21202.

We have previously developed polymerase chain reaction (PCR) methodology for detection of the oyster pathogen, *Perkin-*

sus marinus, from environmental samples and shown it to be sensitive, specific and semi-quantitative with a dilution end-point titer of about 1pg. For more rigorous quantitation, we designed a competitive PCR assay which should be applicable to determination of infection density in oysters and measurement of pathogen concentration in water samples. A competitor fragment was constructed from heterologous DNA of known sequence which was amplified with composite primers consisting of flanking sequence derived from *P. marinus* non-transcribed spacer region of ribosomal RNA gene and internal heterologous sequence. This PCR product produced a competitor fragment which differed in size from the native PCR product but contained ends with *P. marinus*-specific DNA. Dilutions of the competitor fragment were then amplified with *P. marinus*-specific primers in the presence of native DNA, and relative concentrations of competitor vs. target DNA determined by fluorescence imaging of ethidium bromide or Sybr green-stained agarose gels. Spike and recovery assays of oyster tissue seeded with *P. marinus* showed a linear relationship between parasite and DNA concentration and indicated that <10 parasites/15 mg oyster tissue were consistently detectable by competitive PCR. This sensitivity corresponded to <1 pg of *P. marinus* DNA/sample. Using similar methodology, we have also developed competitive PCR for actin and superoxide dismutase sequences which will facilitate studies of gene expression using RT-PCR. Thus, *P. marinus* competitive PCR assays retain sensitivity of the original PCR method and permit rapid assessment of infection density and analysis of potential virulence factors.

DEVELOPMENT OF A PCR ASSAY FOR THE QUANTIFICATION OF PERKINSUS MARINUS. Heather A. Yarnall,* Nancy A. Stokes, and Eugene M. Burrenson, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Routinely, researchers have used Ray's fluid thioglycollate medium culture methods for the detection of the protozoan oyster parasite, *Perkinsus marinus*. While the use of these methods has been fairly effective in monitoring *P. marinus* in oyster tissues, a more sensitive and specific method has been needed to detect *P. marinus* in environmental samples. Immunodetection/flow cytometry methods have been employed to detect this parasite in water samples, but the specificity of the polyclonal antibody has yet to be determined. A molecular approach, however, allows detection with extreme sensitivity and specificity.

The polymerase chain reaction has been utilized to successfully amplify *P. marinus* DNA from infected oyster tissues and hemolymph. Primers have been developed that specifically amplify a region of the ribosomal DNA of *P. marinus*. These primers, however, do not amplify *Peridinium* sp., *Gymnodinium* sp., *Ampelidinium* sp., or *Perkinsus atlanticus* genomic DNA. This assay

allows the detection of 100 fg of DNA on an ethidium-bromide stained agarose gel. Furthermore, as few as 5 *P. marinus* cultured cells can be detected. A study that compared the traditional thioglycollate methods with the PCR assay indicated that the PCR assay comparably detected *P. marinus* cells in oyster tissue.

A mutagenic primer was used to create a competitor molecule which was used as an internal standard in competitive PCR reactions for quantitation. This PCR assay permits quantitation of the *P. marinus* DNA in oyster tissue samples and enumeration of the *P. marinus* cells present in environmental water samples collected in the lower York River during the period from 4/1/94 to 3/31/95. The ability to quantitate *P. marinus* DNA is important for the enumeration of parasites in oyster tissues following laboratory interventions as well as the enumeration of parasites in the water column for transmission dynamics investigations.

REPRODUCTIVE BIOLOGY AND PHYSIOLOGY OF FRESHWATER BIVALVES

OXYGEN CONSUMPTION AND ANAEROBIC METABOLITE CHANGES OF FRESHWATER MUSSELS (UNIONIDAE) FROM DIFFERENT HABITATS DURING DECLINING DISSOLVED OXYGEN AND AIR EXPOSURE. L. Chen,* A. G. Heath, and R. J. Neves, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061–0406.

The oxygen consumption of several species of mussels has been measured under declining dissolved oxygen (DO) levels. The results show that species living in lakes (i.e. *Pyganodon grandis*), pool areas (i.e. *Elliptio complanata*) and bank margins (i.e. *Elliptio fisheriana*) of the river have better ability to regulate oxygen consumption than those found in the riffles (i.e. *Villosa iris* and *Villosa constricta*). The *Villosa iris* is especially sensitive to hypoxia and handling stress. *P. grandis* is tolerant of handling stress and is a good regulator of heart beat under declining dissolved oxygen. The ability to regulate oxygen consumption was improved considerably at lower temperature for *P. grandis* and *V. iris*. For *V. constricta*, the brooding females have higher oxygen consumption and lower ability to regulate oxygen consumption than the males. In other experiments, metabolites of anaerobic metabolism of *E. complanata* and *V. iris* exposed to different DO levels were measured. The results suggest different biochemical mechanisms were used by these two species under hypoxia condition. *E. complanata* produce lactic acid during the short-term hypoxia and produce succinic acid under long-term hypoxia or air exposure. On the other hand, *V. iris* seems to have a lower anaerobic capacity in the current studies.

SALINITY TOLERANCE AND CELL VOLUME REGULATION IN FRESHWATER MUSSELS. Thomas H. Dietz* and Harold Silverman, Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803; Douglas H. Neufeld and Stephen H. Wright, Department of Physiology, University of Arizona, Tucson, AZ 85724.

Freshwater bivalves do not tolerate an acute transfer to a hyperosmotic NaCl medium, and are intolerant of excess potassium in the environment. *Dreissena polymorpha* is the least tolerant being unable to survive a transfer to 45 mM NaCl for more than a few days or 1 mM KCl for a day. However, when transferred to media containing 45 mM NaCl and 1 mM KCl or to 3‰ artificial seawater (SW) the survival is extended for months. Microscopic observation of gill epithelial cells indicate that hyperosmotic 45 mM NaCl added to artificial pondwater (PW) results in rapid (minutes) reduction of cell height in both dreissenid and unionid bivalves, with no evidence of cell volume recovery. If the same hyperosmotic medium contains 1 mM KCl the cells display partial volume recovery. The epithelial cell dimensions are restored to normal values within 30 min when the gills are returned to PW. Hyperosmotic challenge with the addition of NaCl results in $[Na_i] > [K_i]$ within 12 h. In contrast, when K is present in the hyperosmotic medium $[Na_i] = [K_i]$ within 12 h. Thus, freshwater bivalves exposed to hyperosmotic NaCl gain solutes in the extracellular and intracellular (IC) compartments, but are unable to preserve IC volume. The addition of K to the medium without additional NaCl results in cellular swelling. Stoichiometric balance of Na and K in the medium enhances survival.

A REVIEW OF REPRODUCTIVE DIVERSITY AMONG FRESHWATER BIVALVIA AND A CONSIDERATION OF MEDIATING MECHANISMS INVOLVED. William H. Heard,* Department of Biological Science, Florida State University, Tallahassee, FL 32306–2043.

Most freshwater bivalves are eulamellibranch naiades (Palaeo-heterodonta: Unionoida) and fingernail and pill clams (Heterodonta: Veneroida: Sphaeriidae). Animals of the former group display conspicuous vitellogenesis in males (associated with paraspermatozoa but not with subsequent euspermatozoa) as well as in females and hermaphrodites. The animals brood the developing young in at least two of the four demibranchs for about one month (Nearctic summer) or from autumn to late spring or summer. A larval form is discharged, and in most species it is an obligatory parasite on fishes for two–three weeks.

Sphaeriids are also ovoviviparous, producing crawl-away miniatures. Superfetation occurs in *Musculium* and *Sphaerium*, but not in *Byssanodonta* and *Pisidium*. At last some species, all of which have an enormous number of chromosomes, reproduce by syngamy but not fertilization.

Most of the work on these bivalves has been descriptive, and little attention has been given to the environmental cues and mo-

lecular (neurohormonal) mechanisms underlying and mediating the diversity of nominal reproductive strategies.

CHEMOSENSORY ABILITIES OF FRESHWATER MUSSELS AND GLOCHIDIA. William F. Henley* and Richard J. Neves, Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

The ability of gravid freshwater mussels to detect host fish may be important in determining the timing of the release of glochidia. To measure behavioral changes in gravid *Lampsilis fasciola* and *Villosa iris* with exposure to host (*Micropterus dolomieu*) and non-host (*Cyprinus carpio*) fish and fish mucus, a Composite Behavioral Index (CBI) was developed and used in exposure experiments. The CBI was an additive index based on degree of mantle presentation, shell spread (mm), inhalant aperture opening (mm), pulse rate (no./min), and glochidial ejection. Behavioral observations occurred every 10 min. Experiments for *L. fasciola* included exposure to host and non-host mucus and live fish, serotonin, and PGF_{2α}. Experiments for *V. iris* included exposures to host mucus and live fish during day and night observations. *Lampsilis fasciola* was most active during day, while *V. iris* was most active at dusk. For both species, CBI values were greater with exposure to host treatments than controls, and lower than controls in non-host treatments. *V. iris* showed increased responses to exposures within night versus day treatments. Another CBI scoring system was developed to measure the responses of *V. iris* glochidia to host (*M. dolomieu*) and non-host (*C. carpio*) mucus. Exposures to carp blood, carp serum, carp plasma, amino acids, and fibrinogen also were conducted with *V. iris* glochidia. CBI values were higher with exposure to non-host mucus for both species, possibly due to the presence of blood in carp mucus samples. CBI values were highest in fibrinogen treatment exposures, possibly indicating response to chemicals involved in the encystment process rather than to host fish status.

SEASONAL METABOLISM AND BIOCHEMICAL COMPOSITION OF TWO UNIONID MUSSELS, *ACTINONAIAS LIGAMENTINA* AND *AMBLEMA PLICATA*. Daniel J. Hornbach and Shirley M. Baker,* Department of Biology, Macalester College, St. Paul, MN 55105.

We examined the seasonal variation in i) condition index, ii) metabolism, as measured by oxygen uptake, ammonia excretion, O:N ratios, and grazing rates, and iii) biochemical tissue composition (carbohydrate, protein, lipid, and inorganic levels) of the freshwater mussels (Family Unionidae) *Actinonaias ligamentina* (subfamily Lampsilinae) and *Ambelma plicata* (subfamily Ambelminae). Our measurements show that *A. ligamentina* and *A. plicata* have unique seasonal patterns of physiology and biochemical composition. *Actinonaias ligamentina*, a long-term brooder, has a lower condition index, smaller carbohydrate reserves, and greater sensitivity to seasonal changes than does *A. plicata*. *Ambelma plicata*, a short-term brooder, has a higher condition

index, greater carbohydrate reserves, and is affected to a lesser degree by seasonal changes. Our data suggest that species-specific sensitivity to environmental change may have contributed to the decline in diversity of unionid mussels observed in recent decades.

EFFECT OF LIGHT, FOOD AND SILT ON THE TOXICITY OF COPPER TO JUVENILE *LAMPSILIS STRAMINEA CLAIBORNENSIS* MUSSELS. Anne E. Keller,* D. Shane Ruessler, and Nikki Kernaghan, U.S. Geological Survey, 7920 NW 71 Street, Gainesville, FL 32653.

There is no standard acute toxicity test method for unionid mussels. Test methods used to evaluate the toxicity of contaminants to juvenile mussels have been adapted from techniques used with fish and zooplankton. However, mussels occupy benthic habitats of streams and lakes where light is diminished compared to that measured nearer the surface, and where they are exposed to more particulate matter than are pelagic species. A recently published 9-day test method for juvenile unionids requires the use of 24 hour darkness, 800 mg/L silt and a daily dose of an algal mixture. Although the assumption is that these conditions better mimic real-world exposures, and therefore, provide more accurate results than would water-only tests using a 16:8 photoperiod and no food, there are no supporting data. We evaluated the impact of each of these components on the sensitivity of juvenile mussels in a series of toxicity tests with copper sulfate. The presence or absence of light or food made no difference in mussel sensitivity to copper. Little or no difference was detected between LC50s (available copper) calculated for tests that included silt compared to those that did not. Therefore, there appears to be no requirement for darkness, feeding or silt in acute juvenile unionid toxicity tests.

ROLE OF MICROTUBULES IN PRONUCLEAR FORMATION AND MIGRATION AND ESTABLISHMENT OF THE CLEAVAGE PLANE IN FERTILIZED EGGS OF A FRESHWATER MUSSEL, *DREISSENA POLYMORPHA*. John W. Lynn* and J. Rachel Walker, Department of Zoology and Physiology, Louisiana State Univ., Baton Rouge, LA 70803.

The first mitotic spindle is believed to establish the position of the first cleavage plane in many marine zygotes. Microtubules (MT) also have been implicated in pronuclear (PN) formation and migration. No data exists on these processes in freshwater species. In *D. polymorpha*, pronuclear formation and migration are dependent on MTs. When the MT inhibitor demecolcine is introduced before 2nd polar body (PB) formation, neither male nor female PN are formed. Pronuclei form if demecolcine is added after 2nd PB formation, however, PN migration is inhibited. During normal development, the female PN migrates centrally along an axis perpendicular to a tangent drawn to the egg surface at the site of PB formation. In contrast, the male PN migrates toward the female PN along a path varying with the site of sperm entry. Observations of MT distribution made using high resolution DIC microscopy and epifluorescent microscopy with FITC-conjugated monoclonal an-

tubodies to tubulin reveal a bundle of MTs immediately below the site of PB formation and a radiating cone of MTs extended from the bundle toward the female PN. An additional MT array extends from the female PN to the male PN, but no apparent sperm aster is observed. The positioning of the female PN appears to be controlled by the MT anchor and is related to the positioning of the mitotic spindle. Thus, location of the cleavage furrow appears to be dependent upon the positioning of the female PN by the MT anchor and MT array. Supported by Louisiana Sea Grant.

REPRODUCTION IN A FRESHWATER UNIONID (MOLLUSCA: BIVALVIA) COMMUNITY DOWNSTREAM OF CAVE RUN RESERVOIR IN THE LICKING RIVER AT MOORES FERRY, KENTUCKY. Stephen E. McMurray* and Guenter A. Schuster, Department of Biological Sciences, Eastern Kentucky University, Richmond, KY 40475–3124.

One of the most perplexing problems in unionid research is the documented loss of recruitment in some communities (beds). Previous researchers have documented that very little or no recent recruitment has taken place in a diverse unionid bed in the Licking River at Moores Ferry. The objective of this study was to determine why recruitment has decreased or ceased in this bed and to compare it with a healthy community 159 kilometers downstream at Butler, Kentucky. During 1995, collections of unionids, fish, and glochidia were made either bimonthly or monthly. Unionids were checked for gravidity, and five individuals of two target species, *Actinonaias ligamentina* and *Elliptio dilatata*, were returned to the lab for histological examinations. Only 10.1% of the unionids at Moores Ferry and 13.5% at Butler were found to be gravid. Twenty glochidia were found in drift net collections. Six fish, all from Moores Ferry, were found to be infected with glochidia. Temperature and discharge data indicate that large releases from Cave Run Reservoir (35.4 km upstream) during important reproductive periods may impact the unionids at Moores Ferry due to high discharge and corresponding low water temperatures. These data indicate that reproduction is occurring in both communities, but at a drastically reduced rate.

GILL MUSCULATURE IN *DREISSENA POLYMORPHA* AND THE EFFECTS OF ELEVATED IONS. Scott Medler,* Harold Silverman, Thomas H. Dietz, and Cory Thompson, Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

The eulamellibranch gill of *Dreissena polymorpha* is made of a muscle and connective tissue matrix that is covered by regionally specialized epithelial cells. The muscle and connective tissue of the gill work together to determine the shape and dimensions of the passages that allow water flow through the gill. The ionic make up of the Ringer's solution in which excised gills are placed influences contractility of the muscles of the gill. The shape and dimensions of the water passages change spontaneously and in response to exogenous transmitters *in vitro*. Previous studies have

shown that *D. polymorpha* has a marked intolerance for elevated NaCl levels in the bathing medium. Elevated NaCl in the Ringer's solution depresses muscle contractility, but this effect is mitigated when sufficient K⁺ is present in the medium. A ouabain-sensitive transport process is at least in part responsible for maintaining the ion balance needed to maintain muscular activity in the gill.

RESEARCH NEEDS FOR FRESHWATER MUSSELS. Richard J. Neves, Virginia Cooperative Fish and Wildlife Research Unit, Department of Fisheries and Wildlife Sciences, Virginia Tech, Blacksburg, VA 24061–0321.

Our knowledge of the reproductive biology and life history requirements of freshwater mussels (Unionidae) has advanced significantly during the last decade, perhaps in time to prevent the extinction of most of the 58 federally endangered species. Host fishes have now been identified for roughly one-third of the fauna, and numerous studies are underway to evaluate the suitability of artificial environments to sustain broodstock for propagation. However, the exotic zebra mussel, *Dreissena polymorpha*, continues to spread, causing mortalities of native mussels in commercial and rare populations throughout the central U.S. Methods for controlled spawning, juvenile culture, and reintroduction to historic habitats are being tested, but they are inadequate at this time to address the upcoming crisis. The urgency of perfecting culture techniques mandates cooperation by mariculturists to provide expertise on techniques and technology for mussel propagation. Freshwater mussel biologists must tap the information base in shellfish mariculture to expedite preparations for a national effort to prevent a pending spasm of unionid extinctions unmatched in modern times.

POPULATION DYNAMICS OF *LEPTODEA FRAGILIS* IN A ZEBRA MUSSEL-INFESTED LAKE ERIE WETLAND. S. Jerrine Nichols,* Great Lakes Science Center, U.S. Geological Survey, 1451 Green Rd., Ann Arbor, MI 48105; Jon Amberg, Fisheries and Wildlife Department, Michigan State University, East Lansing, MI.

In 1996, several thousand *Leptodea fragilis* were collected during the dewatering of a zebra mussel infested marsh located in the western basin of Lake Erie. Less than 1% of the population showed any sign of recent or past zebra mussel colonization. Multi-year classes of *L. fragilis* were found, ranging in shell length from 17–169 mm, and in age from 1–10 years (as determined by shell sections). Seventy-one percent of the population was 51–80 mm in shell length, but ranged in age from 2–4.5 years. Growth rates were not consistent between individuals or between years. For example, some individuals grew rapidly, reaching 105 mm in 3.5 years, while others only grew 45 mm in 3.5 years. Some individuals, but not all, showed years of minimal growth interspersed in between years of rapid growth. Sexual maturity and shell sexual dimorphism were first observed in shells 41–50 mm in length and females in general, were more common than males (2:1 ratio). The

strong recruitment and rapid growth indicates that the presence of zebra mussels had only a minimal impact on the existing *L. fragilis* population.

COEXISTENCE OF ZEBRA MUSSELS AND NATIVE CLAMS IN A LAKE ERIE COASTAL WETLAND. S Jerrine Nichols* and Douglas Wilcox, Great Lakes Science Center, USGS, 1451 Green Rd., Ann Arbor, MI 48105.

Native clam populations can coexist with zebra mussels in specialized habitats such as wetlands. In 1996, 22 native clam species were discovered and relocated from a coastal wetland, Metzger Marsh, just west of Toledo Ohio. Large numbers of zebra mussels of several year classes were colonizing parts of this marsh. Multiple size classes of native clams were also collected for 18 of the 22 species; e.g., *A. plicata* (6 year classes, size range 10–147 mm), *Lampsilis r. luteola* (3 year classes, size range 47–123 mm). Many of the clams, such as *A. plicata* and *L. fragilis* were gravid when collected. Less than 1% of the 7,000 clams collected showed any degree of zebra mussel colonization, either by actual mussels or remnant byssus threads. Laboratory tests indicate that zebra mussel colonization of the native clams was probably limited due to the soft sediments and high water temperatures characteristic of this wetland habitat encouraging burrowing. The survival and continuing successful reproduction of native clams at this Lake Erie wetland indicates that such sites may provide additional refugia for native clam populations.

GUT CONTENTS OF UNIONIDS FROM THE ZEBRA MUSSEL INFESTED OHIO RIVER, AND FROM ZEBRA MUSSEL-FREE POND REFUGIA. Bruce C. Parker,* Catherine M. Gatenby, and Matthew A. Patterson, Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

Gut contents of unionids collected from areas of the Ohio River with high and low zebra mussel (*Dreissena polymorpha*) infestation and from unionids held in zebra mussel-free pond refugia in Leetown, WV were examined to identify and quantify algal genera. Algae from the Ohio River and pond refugia also were identified and quantified. Unionids not sacrificed in the field were cleaned and transferred to a quarantine facility for a minimum of 4 weeks prior to transport to zebra-free refugia; gut contents of unionids from the river, from quarantine, and from specimens held in pond refugia for over 1 yr were compared.

The gut contents of mussels revealed much detritus and a wide variety of unicellular, colonial and filamentous algae, which included mostly diatoms, green algae, and bluegreen algae. Algal cells ranged 5–100 μm , the 100 μm for filaments. Cell numbers in the guts ranged 10^4 – 10^6 cells/mL except for mussels held at least one week in quarantine without food which had no detectable algae in their guts. Interestingly, the density of algae in the Ohio River and in the ponds also ranged 10^4 – 10^6 cells/mL. Unionids from ponds expectedly had gut contents similar to the pond plank-

ton; however, diatoms often were found in greater proportion in the guts than in the pond plankton. Apparently feeding is relatively non-selective as a wide variety of plankton are ingested. Most algal genera in the Ohio River and the ponds, and those ingested by the mussels are true plankton forms. Over 66% of the algal genera in the ponds also are known to the Ohio River.

USE OF GLYCOGEN LEVELS TO ASSESS THE GENERAL HEALTH OF UNIONIDS FROM THE ZEBRA MUSSEL INFESTED OHIO RIVER AND FROM QUARANTINE. Matthew A. Patterson,* Bruce C. Parker, and Richard J. Neves, Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

During the summer of 1996, 500 specimens of *Amblema plicata* and *Quadrula pustulosa* were collected from the Ohio River. Ten specimens of each were sacrificed in the field from areas of low zebra mussel (*Dreissena polymorpha*) infestation (0.3 zebra mussels/ m^2) and high zebra mussel infestation (>300 hundred zebra mussels/ m^2). Mussels not sacrificed in the field were transported to a quarantine facility and sacrificed at the end of one week, two weeks and four weeks prior to transport to pond refugia. Mussels were not fed to determine the impacts of starvation during quarantine. After preservation in 95% ethanol, glycogen levels were determined by homogenizing mantle tissue in perchloric acid, reacting with the enzyme amyloglucosidase and reading at 450 nm with a Beckman DU640 spectrophotometer.

Mean glycogen levels (mg glycogen/g wet weight tissue) of *A. plicata* from the high infested site were significantly lower (2.73 mg/g) than those from the low infested site (8.08 mg/g) ($p = 0.05$). Glycogen levels also dropped significantly at the end of one week of quarantine ($p = 0.05$). After stabilizing at the end of week two, glycogen levels again dropped after four weeks of quarantine ($p = 0.05$). Glycogen levels of *Quadrula pustulosa* followed the same pattern except that significant declines in glycogen levels were not observed until the fourth week of quarantine.

The data show that increased zebra mussel infestation and starvation during quarantine may result in significant reductions in energy stores of native unionids. Although for conservation purposes it may be necessary to relocate native unionids from zebra mussel infested waters, proper feeding during quarantine may be essential to ensure the continued survival of unionids after transport from quarantine.

SURVIVAL OF JUVENILE UNIONID MUSSELS CULTURED UNDER SEVERAL FOOD AND WATER REGIMES. D. Shane Ruessler and Anne E. Keller, U.S. Geological Survey, 7920 NW 71 Street, Gainesville, FL 32653.

The culture of unionid mussels is an important pre-requisite to reintroduction activities. While many species can now be cultured through the larval stage by *in vivo* or *in vitro* methods, there has been only limited success in maintaining and growing the juveniles beyond a few months. Several researchers have failed in attempts

to culture juvenile mussels for six months to a year with acceptable survival (~50% or greater). We used a fairly simple method that combines daily feeding, silt substrate and flow-through well water to culture five species of mussels, *Uterbackia imbecillis*, *Villosa villosa*, *Lampsilis teres*, *Lampsilis staminea claibornensis* and *Epioblasma triquetra*. Survival varied by species and among various food, substrate and water combinations. Species survival vs habitat preferences and culture conditions will be discussed, as well as the potential of this method to produce large numbers of juvenile unionids for reintroduction or experimental purposes.

FACTORS GOVERNING THE DISTRIBUTION, ABUNDANCE, GROWTH AND REPRODUCTION OF THE FRESHWATER MUSSEL, *MARGARITIFERA FALCATA*, IN FORESTED WATERSHEDS OF WESTERN WASHINGTON. Kelly Toy,* Washington Cooperative Fish and Wildlife Research Unit, School of Fisheries, Box 357980, University of Washington, Seattle, WA 98189–7980.

Margaritifera falcata (family: Margaritiferidae) is the most common freshwater mussel species found in forested watersheds in western Washington. Currently there are no existing studies or population estimates for this species in western Washington. A survey was conducted in Battle Creek, located on the Tulalip reservation and Bear Creek, near Woodinville, Washington. Growth rate and population age structure was determined for both streams by using an internal ageing method. Mussel density in Battle Creek was found to be 84 mussels per m² and 55 mussels per m² in Bear Creek. Growth rates for both mussel populations were found to be similar. Histological analysis was performed to identify gametogenic stages, mode of reproduction, and time of glochidia release. A correlation between water temperature and spawning was determined using temperature recordings over a one year period. Characterization of mussel habitat include substrate, current velocity, and water chemistry analysis.

METAMORPHOSIS OF FRESHWATER MUSSELS ON HOSTS IN CAPTIVITY. G. Thomas Watters,* Ohio Biological Survey and Aquatic Ecology Laboratory, Ohio State University, Columbus, OH 43212; Scott H. O'Dee, School of Natural Resources, Ohio State University, Columbus, OH 43212.

With the current efforts to remove mussels to hatcheries and other enclosures to avoid mortality from zebra mussels, it was important to determine if mussels can reproduce while in captivity. Two host-mussel populations were established in outdoor enclosures. The first enclosure contained twenty hatchery-raised largemouth bass and twenty threeridge, *Amblema plicata*. The sex ratio of the mussels was not determined. The second enclosure contained twenty hatchery-raised largemouth bass and ten male and ten female fatmuckets, *Lampsilis radiata siliquoidea*. Enclosures were 3028 liter tubs with a 1.8 m base diameter. No effort was made to control water chemistry or temperature. Mussels were kept in sediment-filled containers and fed a tri-algal diet. Glochidia

samplers were placed on the bottom and examined every two days. After a year, an estimated 102 metamorphosed juveniles (0.75% of all glochidia recovered) were produced in the *Lampsilis* pool in May and June. This indicated that recruitment in a hatchery setting was possible using passive methods—without handling of fishes or mussels. No metamorphosed juveniles were produced in the *Amblema* pool. Although *Lampsilis radiata siliquoidea* released glochidia year-round, most were released at water temperatures of 13°C and above (May to August), and consisted of numerous “peaks” or releases. Maximum release occurred at 20°C. Only a single peak of glochidial release was seen for *Amblema plicata*. This occurred when water temperature reached 20°C (July).

SCALLOPS: PROBLEMS AND SOLUTIONS

BAY SCALLOP RESTOCKING EFFORTS IN SARASOTA BAY, FLORIDA, THROUGH THE USE OF TRANSPLANTED SPawner STOCKS. Jay R. Leverone,* Mote Marine Laboratory, Sarasota, FL 34236.

A project to reestablish bay scallops (*Argopecten irradians concentricus*) in Sarasota Bay, Florida, was conducted from October, 1993 through July, 1994. Spawner stock were transferred from coastal seagrass meadows near Steinhatchee, FL in late September, 1993, to a protected embayment (Pansy Bayou) in Sarasota Bay. The transplant was scheduled to coincide with the seasonal spawning cycle of Florida bay scallops. Six hundred fifty adult scallops were maintained in cages and monitored for growth, survival and reproductive condition. Spatfall and recruitment were monitored within Pansy Bayou and adjoining seagrass meadows during the subsequent winter and spring. A continuous record of water temperature was also maintained.

Several abrupt drops in temperature occurred toward the end of October. After a period of high mortality (25.4%) on Nov 09, mortality remained low throughout the winter. A few scallops survived through the following spring (Jun 04). Spatfall was observed only within Pansy Bayou. Juvenile and subadult abundance the following spring was highest within Pansy Bayou (73% of total collected).

The overall limited number of spat, coupled with low spawning activity and prolonged survival of scallops through the winter, suggest that, although environmental conditions were favorable and transplanted scallops were poised to respond, a major spawning event did not occur in Pansy Bayou.

COMPOSTING CALICO SCALLOP PROCESSING RESIDUES, AN ALTERNATIVE DISPOSAL OPTION. William T. Mahan, Jr., University of Florida/Franklin County Extension Program, 33 Market Street, Suite 305, Apalachicola, FL 32320–2310.

During the initial development (1970) of the calico scallop (*Aequipecten gibbus*) industry in Florida, production was limited

and waste disposal was not an issue. However, development of a mechanical shucking process in 1974, resulted in increased scallop production. By 1984, statewide production peaked at 26.3 million pounds of meats and the Department of Environmental Regulation became concerned about odor and pollution problems associated with inshore and nearshore disposal of the processing wastes. To alleviate this concern, some processors began sending processing residues to landfills.

Then in 1988, the Florida Legislature passed the Solid Waste Management Act (SWMA) which mandated major changes in solid waste management practices. Brevard and Franklin were two of the five counties specifically identified in the Act as having major seafood processing waste disposal problems. In fact, the problem got so bad in Franklin County that in May 1990, the Board of County Commissioners passed a resolution prohibiting the disposal of seafood processing wastes at their new landfill, leaving the industry with limited legal disposal options, ocean dumping and trucking the waste out of county.

To help these counties and the scallop industry solve their waste problems, the University of Florida's Sea Grant Program worked cooperatively with county officials and seafood processors (Brevard 1991 & Franklin 1993) to develop scallop viscera compost operations as a solution to their landfill problems.

REPRODUCTION, RECRUITMENT, AND ADAPTIVE STRATEGIES OF BAY SCALLOP POPULATIONS: A HOUSE OF CARDS? Dan C. Marelli and William S. Arnold, Florida Department of Environmental Protection, Florida Marine Research Institute, 100 8th Avenue SE, St. Petersburg, FL 33701.

Many bay scallop populations along the Florida Gulf coast have drastically declined during the past 5–30 years. These populations once supported commercial and recreational fisheries but are now virtually extirpated. Researchers theorize population crashes have been induced in part by habitat destruction, overharvest, and declining water quality. The relationship between spawner-stock abundance and recruitment in scallops has traditionally been portrayed as effectively nonexistent, leading some researchers to question whether overharvest can significantly reduce the recruitment potential of a scallop population. Anecdotal evidence suggests that overharvest has indeed severely impacted some populations, and that biologists must consider a new stock-recruitment paradigm.

Particularly troubling to resource managers are populations that have failed to recover after the removal of harvest effort. Most of these populations persist at very low densities, and we predict that fertilization success in such depauperate populations is very low. It is possible that self fertilization is the major mode of reproduction in these populations. Data from Florida Gulf coast bay scallop populations during 1993–1995 demonstrate that coherence between recruitment and stock abundance is low but more apparent at low stock abundances. Recruitment failure may be a regular feature of low stock abundances and, combined with histological

reproductive staging, seems to provide evidence for two life history strategies that combine to produce persistent scallop populations. Spawning during the late summer occurs at a low level and produces similarly low levels of recruitment. Following the major decline in water temperature in early October, a catastrophic spawning event that produces large numbers of recruits is typically seen in abundant populations and is less evident in depauperate populations.

Our data suggest that populations may experience depensation at low abundances and that recruitment failure, typically invoked to explain lack of stock persistence, may be more closely related to reproductive failure than previous researchers have realized. Long-term population stability may be guaranteed by low-level asynchronous spawning, while large exploitable populations are produced by more risky catastrophic spawning events.

THE REPRODUCTIVE BIOLOGY OF THE CALICO SCALLOP, *ARGOPECTEN GIBBUS* (LINNAEUS). Michael A. Moyer* and Norman J. Blake, Department of Marine Science, University of South Florida, Saint Petersburg, FL 33701.

The calico scallop, *Argopecten gibbus* (Linnaeus), forms the basis of an important commercial industry on the east coast of Florida. Large fluctuations in annual landings reflect the large variability in the stock availability for this short lived species. These fluctuations are due, at least in part, to spawning success which in turn is influenced by several environmental factors. The lack of sufficient information on the reproductive biology of this animal prompted this research. Field samples were collected over a 12 year period and the reproductive state of the animals determined. The reproductive cycle as determined through body component indices, body component weights, histological staging and oocyte measurements is presented. This provides a clear picture of the reproductive biology of this animal as well as providing a comparison of the various techniques used to examine the reproductive state of marine bivalves. This information is related to field measurements of various parameters including temperature and phytoplankton concentration. This information is supplemented with laboratory studies on how the reproductive biology of the calico scallop is influenced by temperature and food abundance.

LARVAL SURVIVAL OF THE ROCK SCALLOP, *CRASSADOMA GIGANTEA* IN THE HATCHERY. Walter Y. Rhee,* The Seafood Advisor, P.O. Box 658, Bedford, MA 01730; Jonathan P. Davis, Taylor Resources, Inc., 701 Broadspit Road, Quilcene, WA 98376.

Over the past two decades, numerous attempts have been made on the U.S. West Coast to produce rock scallop (*Crassadoma gigantea*) seed in the hatchery. However, inconsistent larval development and metamorphosis hampered commercial hatchery seed production. Earlier studies on the inconsistent spawning and larval growout indicate: 1) Poorly defined spawning cycles 2) the energy for larval metamorphosis coming from endogenous energy

reserves that the broodstock passed down to the eggs rather than from the exogenous energy the scallop larvae obtained from grazing. A mixed diet of 15,000 + cells/ml of *Chaetoceros calcitrans*, *Skeletonema* sp., *Tahitian isochrysis*, *Thalassiosira* sp., plus *Rhodomonas* sp. were fed 7 to 8 liters/day to each rock scallop broodstock (12 females, 3 males) for 4 weeks during the non-spawning season (October 1996 to November 1996). Spawning was induced by injecting serotonin ($0.2 \text{ ml} \times 10^{-4} \text{ M}$) into the adductor muscle and the sperm and eggs were mixed to produce larvae. Consistent spawning and larval survival were obtained from the ongoing experiment. High numbers of mature eggs and high larvae survival were repeatedly obtained during a non-spawning season when gonads normally undergo atresia.

THE INFLUENCE OF TEMPERATURE ON SPAWNING AND SPAT COLLECTION OF THE BAY SCALLOP, *ARGOPECTEN IRRADIANS* IN SOUTHEASTERN MASSACHUSETTS WATERS, USA. Karin A. Tammi* and Wayne H. Turner, The Water Works Group, Inc., P.O. Box 197, Westport Point, MA 02791; Michael A. Rice, Department of Fisheries Animal and Veterinary Science, University of Rhode Island, Kingston, RI 02881.

During the past four years, research of the Bay Scallop Restoration Project has been tracking the spawning time, and settlement of the bay scallop, *Argopecten irradians* to artificial spat collectors along the southeastern coast of Massachusetts. The goal of this research has been to enhance scallop populations by using spawning sanctuaries and artificial spat collectors. One of the objectives of this research has been to optimize the deployment time of collectors by monitoring water temperature, gonadal maturation and bivalve larval abundance. Initiated during the summer of 1993 in the Westport River, this research has recently expanded to include the waters of Apponagansett Bay in Dartmouth, Massachusetts. Researchers have been able to accurately determine the spawning and settlement events of *A. irradians* which usually spawns heavily in late June. However, in 1996 a notable departure from three years of data was recorded in regard to water temperatures, spawning and settlement. As a result, larval monitoring detected the spawning time for all shellfish to be six weeks later than expected with the greatest recruitment to artificial collectors for all study areas occurring between late August and mid September. Furthermore, spat from the 1996 season was considerably smaller with a shell height under 15 mm compared to average spat size from 1993 to 1995 which ranged from 24 mm to 37 mm. The data obtained from the 1996 study indicates that the spawning of *A. irradians* was delayed by the cooler water temperatures with the heavy spawn occurring in August. More importantly this data indicates that 1996 seed are a much smaller size than previous years making winter survival speculative. This temperature event also sheds light on why bay scallop crops experience such drastic annual population fluctuations.

TOXICANTS/TOXINS AND SHELLFISH

THE EFFECTS OF ENVIRONMENTAL STRESSORS ON DEFENSE MECHANISMS AND PROGRESSION OF *PERKINSUS MARINUS* INFECTIONS IN *CRASSOSTREA VIRGINICA*. Robert S. Anderson* and Lisa L. Brubacher, Chesapeake Biological Laboratory, University of Maryland, Solomons, MD 20688; Lisa M. Calvo, Michael A. Unger, and Eugene M. Bureson, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Certain environmental contaminants inhibit putative immune responses of oyster hemocytes after in vitro exposure. This suggests that pollutant exposure may exacerbate the intensity or progression of oyster diseases in the field. In fact, studies have shown that infection by the protozoan parasite *Perkinsus marinus* is enhanced by environmental chemicals such as tributyltin (TBT) and polycyclic aromatic hydrocarbons, as well as other stressors such as hypoxia. Recently attempts were made to determine if immunomodulation could account for this apparent chemically-mediated alteration of disease resistance. Total hemocyte count and hemocytic defense responses (phagocytosis, bactericidal activity, and oxyradical production via chemiluminescence, cytochrome c reduction, and nitroblue tetrazolium reduction), as well as serum lysozyme levels were followed during the course of both natural and experimental *P. marinus* infections in control oysters and in those exposed to TBT or hypoxia. Hemocyte recruitment into the circulation and increased oxyradical responses of the hemocytes were seen in all oysters with advanced infections. However, it was difficult to detect immunomodulation that was solely associated with exposure to the environmental stressors, i.e. to establish a direct link between disease progression and immunosuppression.

LIPID CLASS COMPOSITION OF OYSTERS, *CRASSOSTREA VIRGINICA*, EXPOSED TO SEDIMENT-ASSOCIATED PAHs. Fu-Lin E. Chu,* Tong Li, Aswani Volety, Georgeta Constantin, and Robert C. Hale, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Lipids are involved in a number of essential growth and reproductive processes in marine organisms. Impairment of lipid metabolism could cause adverse effects on the above processes. Two experiments were conducted to investigate the effects of PAH-adsorbed sediment on lipid class composition in the eastern oyster, *Crassostrea virginica*. Oysters were exposed to two doses of sediment (60 µg and 120 µg PAHs/oyster) containing a mixture of fluoranthene, pyrene, chrysene, benzo[ghi]perylene, benzo[a]pyrene and benzo[e]pyrene, 4x/week in Experiment 1, and 5x/week in Experiment 2. Control oysters were exposed to non-PAH adsorbed sediment. Results suggest that PAH-exposure modulated the oyster lipid mobilization in Experiment 1. While control oyster plasma total lipid (TL), phospholipid (PL), and sterol were relatively

stable throughout the experiment, PAH-exposure reduced the plasma TL, PL, and sterol after 19 days of exposure (DE). Then, these three lipids increased to a level similar to control at 34 DE in Dose 2 oysters and 46 DE in Dose 1 oysters. Long-term exposure appeared to stress the oysters, and the lipid levels in both Dose 1 and 2 oysters declined at 108 days of exposure. However, this was not the case in the Experiment 2 oysters expressing reproductive activity. No significant change was found in plasma TL, PL and sterol in PAH-exposed oysters over exposure time in this experiment. No general trend of changes was noticed in the lipid contents in oyster tissues and any significant effect of PAH-exposure was found in tissue lipid contents in either experiment. Currently, we are examining the lipid class composition of oysters exposed to sediments collected from PAHs contaminated sites.

REPRODUCTIVE PATHOLOGY IN THREE INDIGENOUS POPULATIONS OF THE BLUE MUSSEL, *MYTILUS EDULIS*, FROM BOSTON HARBOR AND CAPE COD BAY.

Deirdre M. Kimball,* National Oceanic and Atmospheric Administration, National Marine Fisheries Service, One Blackburn Drive, Gloucester, MA 01930.

Previous research has suggested that U.S. East Coast mussels are in poorer condition than indigenous populations on the West Coast (Yevich and Barszcz 1983). To examine this hypothesis in terms of reproductive integrity, mantle tissue from subtidal populations of *Mytilus edulis* at two contaminated National Status and Trends sites in Boston Harbor and a less polluted population in Cape Cod Bay (MA) was evaluated over twenty consecutive months. Three criteria were used to assess relative pathology in histological sections: Hemocytic status (granulomas, hemocytosis), parasitism, and overall tissue health. Parasitism and pathology were ubiquitous temporally as well as spatially. Statistically significant differences were observable only within-site, between sexes. Higher incidences of pathology and parasitism were found in females than in males at the three sites. The extensive gonadal pathology, especially extensive oocyte atresia, observed throughout the annual reproductive cycle, suggests a diminished reproductive capacity within these three populations during 1989 and 1990.

THE ROLE OF HARMFUL ALGAL BLOOMS IN SHELLFISH DISEASE. Jan H. Landsberg, Florida Marine Research Institute, Florida Department of Environmental Protection, 100 Eighth Ave S.E., St. Petersburg, FL 33701–5095.

The role of harmful algal blooms both in shellfish poisonings in humans and in causing mass mortalities of aquatic organisms is well documented. Filter-feeding bivalves accumulate microalgal biotoxins which in turn become available to consumers, both animal and human, through the food chain. In some molluscan species, the presence of harmful algal blooms has been demonstrated to lead to acute behavioral, physiological, or pathological re-

sponses, and, in some cases, mortalities. There is little information concerning chronic, lethal or sublethal effects on shellfish caused by bioaccumulated or biomagnified algal toxins nor whether such effects render shellfish susceptible to disease. The potential for some biotoxins to act as immunomodulators has not yet been explored. A recent review of the literature indicates a strong, though circumstantial, association between the presence of certain types of accumulated biotoxin components in shellfish and the distribution of two common types of bivalve neoplasia. In some cases, disseminated neoplasia and germinomas are highly associated with biotoxin components on both a temporal and spatial basis. No experimental assays or field monitoring studies to investigate or corroborate these relationships have yet been done. In addition to the potential for neoplastic induction in shellfish, toxic microalgal blooms may also precede or coincide with some unexplained mass mortalities or disease phenomena. Conversely, diseased or parasitized shellfish may be more susceptible to, and further weakened by, harmful algal bloom exposure. Bacterial pathogens such as *Vibrio* spp. are often associated with harmful algal blooms. Such bacteria are well known as pathogens in shellfish that are not exposed to harmful algal blooms. However, the potential significance of bloom-associated bacteria as etiological agents of disease when shellfish are exposed to these blooms is unknown. The linkages between chemical contaminants and their association with neoplasia or disease susceptibility in shellfish have been relatively well researched. However, studies of the epizootiology of disease and neoplasia in shellfish should also take into account environmental factors, particularly the distribution of harmful algal blooms, and a potential correlation with accumulated biotoxins.

OBSERVATIONS ON THE HISTOPATHOLOGY OF BAY MUSSELS, *MYTILUS TROSSULUS*: OIL ASSESSMENT STUDIES IN PRINCE WILLIAM SOUND ALASKA. J.

Frank Morado* and **Lisa L. Mooney**, National Oceanic & Atmospheric Administration, National Marine Fisheries Service, Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle, WA 98115–0070.

On 24 March, 1989, the supertanker *Exxon Valdez* ran aground Bligh Reef in Prince William Sound (PWS), Alaska spilling approximately 258,000 barrels (11 million gallons) of crude Alaska North Slope oil. In the days that followed, oil from the spill was directed southwest past Knight Island, PWS and into the Gulf of Alaska by the prevailing wind and surface water current. Once in the Gulf of Alaska, the oil continued southwest, contaminating beaches along the Alaskan Peninsula, Kenai Peninsula and the Kodiak Archipelago. Overall, more than 1100 km of coastline was contaminated by the oil.

This study was initiated to contrast, on a very limited scale, the overall condition of Prince William Sound reference mussels, *Mytilus trossulus*, to those chronically exposed to residual oil from the *Exxon Valdez* oil spill. The mussel collections were from a

single, focal sampling period which limits the ability to draw clear causal relationships. Recognition of this problem necessitated an evaluation of the overall fitness of collected mussels, therefore, both normal and pathological observations were recorded so that the general condition of mussels from oiled and unoiled sites could be determined and compared. In general, non-specific hemocytic infiltrates, brown cell aggregates and digestive gland metaplasia were more prevalent in mussels from heavily oiled sites than from control specimens. In contrast, nutrient storage cells were less prevalent in heavily oiled mussels than in mussels collected from control sites. These results suggest that mussels are still affected by residual oil, four years after the spill.

ASSOCIATION OF TRACE METAL BURDENS WITH HEMOCYTE ACTIVITIES IN OYSTERS FROM TAMPA BAY, FLORIDA. Leah M. Oliver* and William S. Fisher, U.S. EPA National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, 1 Sabine Island Drive, Gulf Breeze, FL 32561–5299.

During winter 1993, eastern oysters (*Crassostrea virginica*) were collected from 16 sites in Tampa Bay, Florida, that receive a range of pollutant types and amounts. Several physiological and defense-related measurements were collected from the oysters in order to evaluate their utility as potential biomarkers of xenobiotic exposure. Contaminant concentrations in oyster tissues were also measured in a composite tissue sample of 20 oysters from each site. Several measurements of oyster hemocyte activity (hemocyte number, percent mobility, rate of locomotion, phagocytic activity and superoxide anion (O_2^-) producing ability showed elevated levels at sites where high tissue burdens of heavy metals were also present. The specific metals that seemed to be associated with increased hemocyte activities included barium, copper, iron and zinc, all divalent cations that may be sequestered within hemocytes. It is possible that accompanying the sequestration of these materials by oyster hemocytes, measurable elevation in certain functions occurs, providing a potential tool by which sublethal deleterious effects can be detected in oysters.

CHEMICAL AND OTHER STRESSORS IN THE GULF OF RIGA: INTERPRETING MULTIPLE LESIONS IN THE CLAM *MACOMA BALTHICA*. Esther C. Peters,* Tetra Tech, Inc., 10306 Eaton Place, Suite 340, Fairfax, VA 22030; Kari K. Lehtonen, Finnish Institute of Marine Research, P.O. Box 33, FIN-00931, Helsinki, Finland.

The Gulf of Riga has been the focus of an international multidisciplinary project during the last several years. A semi-enclosed bay in the Baltic Sea, the gulf receives large amounts of nutrients, particulate matter, and potentially toxic chemical contaminants (especially organics and heavy metals) from an extensive watershed. The city of Riga, near the mouth of the Daugava River, is a

significant point source of pollution, resulting in considerable eutrophication of the gulf. Several studies were conducted to determine concentrations of nutrients and contaminants in water and sediment. The clam, *Macoma balthica*, was sampled throughout the gulf to examine its biochemistry and histopathology in relation to pollution. Lipids, instead of glycogen, were important for energy storage in the clams from the gulf. Histopathological examinations of approximately 500 clams collected quasi-seasonally between July 1993 and May 1995 revealed degenerative and inflammatory changes in the kidney, pericardial gland, heart, and digestive gland. Neoplasms of possible hemic and germ cell origin were found in clams from the station nearest the river. An unknown protozoal parasite appeared to alter the basophilic cells of the digestive diverticula in many clams collected during the study. This research, in addition to other recent data on bivalve toxicology, provided important insights for the interpretation of sublethal effects in benthic organisms exposed to chronic sediment contamination.

EFFECTS OF HARMFUL AND TOXIC ALGAL BLOOMS ON SHELLFISH. Sandra E. Shumway, Natural Science Division, Southampton College, Southampton, NY 11968.

Blooms of toxic and harmful algae occur worldwide and their frequency and distribution are increasing. In some regions, these blooms are responsible for mass mortalities of shellfish—either as a result of direct toxicity, anoxia, or effects on the gills of filter-feeding organisms. In other cases, the effects of these blooms are sublethal. The most common effect is a decrease in exposure to the environment either by reduced filtration, or increased periods of valve closure. Other physiological effects noted, such as changes in oxygen consumption and cardiac activity, may be associated with the former and may not be a direct effect of exposure to harmful or toxic algae. It has also been shown that certain species of commercially important shellfish accumulate toxins and retain them for extended periods of time (>4 years).

Effects of these harmful algal blooms on shellfish will be summarized with respect to potential impacts on the shellfish and aquaculture industries.

EFFECTS OF BARIUM EXPOSURE ON FERTILIZATION AND DEVELOPMENT IN THE WHITE SEA URCHIN (*LYTECHINUS ANAMESUS*). Jill V. Spangenberg* and G. N. Cherr, Bodega Marine Laboratory, University of California Davis, P.O. Box 247, Bodega Bay, CA 94923.

The divalent metal barium (Ba) commonly occurs in high concentrations in produced water (PW), a complex waste associated with hydrocarbon extraction activities. When extraction occurs in marine locations, large volumes of PW are typically discharged into the immediate environment. This has generated concern about, and investigation of, potential adverse biological effects

associated with such discharges. The majority of toxicity associated with a particular PW waste was previously associated with the cationic Ba component. We have previously found subacute exposure to environmentally relevant levels of Ba in sea water (250–900 ppb) to adversely affect veliger development in mussel larvae. Current work involves chronic static renewal bath exposure of adult white sea urchins (*Lytechinus anamesus*) to these same environmentally relevant concentrations of Ba in sea water. Results indicate that adults so exposed exhibit apparent decreased fertilization success and impaired development of progeny, despite the fact that the progeny themselves are not exposed to Ba directly. Impairment of early fertilization events, delay of later developmental events, and abnormal larval calcification suggest perturbation of critical ionic mechanisms in gametes and developing larvae. These findings concur with previous field and laboratory results from investigations of exposure to whole PW in a variety of organisms.

EFFECTS OF PAHs ON THE FUNCTION OF HEMOCYTES FROM EASTERN OYSTERS *CRASSOSTREA VIRGINICA*.

Aswani K. Volety,* Fu-Lin E. Chu, Georgeta Constantin, and Robert C. Hale, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

Hemocyte activities were assessed in oysters exposed to sediment sorbed PAHs (a mixture of fluoranthene, pyrene, chrysene, benzo [e] pyrene, benzo [a] pyrene, benzo [ghi] perylene). Daily doses of 0 or 30 µg/oyster for 60 days (Experiment 1) and 0, 60, or 120 µg/oyster three times/week for 41 days (Experiment 2) were used. *In vitro* effects of water soluble fractions (WSFs) prepared from sediment collected from a heavily polluted area (Elizabeth River, Virginia) on the hemocyte activities were also assessed. In Experiment 1, 30 days of exposure to PAHs reduced the hemocytes' ability to incorporate ³H-labeled thymine, uridine and leucine. After 60 days of exposure, the overall uptake of these three components by hemocytes declined in both control and PAH-S exposed oysters and no significant difference between control and PAH-S exposed oysters was noted. However, after 60 days of exposure, phagocytic, chemotactic and chemiluminescence responses were significantly lower in hemocytes from PAH exposed than from control oysters. In contrast to Experiment 1, no difference was noted in ³H-thymidine, uridine and leucine incorporation in hemocytes between control and PAH-S exposed groups 14 and 30 days after exposure. The uptake of these compounds increased at the end of the experiment in all groups including controls. Phagocytosis did not differ between treatments, nor change with exposure time. No difference was observed in chemiluminescence measured at the end of 41 days among treatments. *In vitro* exposure of hemocytes to 30 and 50% WSFs significantly reduced chemotaxis, phagocytosis, and chemotaxis while stimulating mitochondrial dehydrogenases production.

POSTER SESSION

RESEARCH EXPERIENCES FOR MINORITIES IN MARINE AND ENVIRONMENTAL SCIENCE. **Charles A. Barans, M. Yvonne Bobo, and Donnia L. Richardson,*** South Carolina Department of Natural Resources, Marine Resources Division, Charleston, SC 29412.

As society's cultural, ethnic and racial diversity increases, it becomes increasingly important to ensure that minorities are adequately represented in the sciences. The South Carolina Department of Natural Resources—Marine Resources Division is making a contribution toward this end by increasing minority representation in the sciences and by expanding the number of undergraduates choosing marine and environmental sciences as a profession. The Marine Resources Division initiated an internship program in 1990, which has allowed minority students the opportunity to participate in its diverse research activities.

In 1996, the National Science Foundation's Ocean Sciences Program funded a three-year summer training project based upon the Marine Resources Division's proven record of high quality research, teaching and training experience, established minority support groups, research and academic collaborations, and enthusiastic scientific and administrative staff. The 12-week project involves students in mentor-assisted, independent research projects and provides training in the basic principles of marine science and scientific inquiry.

During the first funding year (1996), three students from Texas, North Carolina and South Carolina, participated in non-vertebrate scientific research. As part of the requirements for the project, students submitted a written summary of their projects and orally presented their findings to other researchers. The program offers housing and provides a stipend for the students.

A PRELIMINARY OVERVIEW OF *PERKINSUS MARINUS* IN SOUTH CAROLINA OYSTER POPULATIONS 1972–1996. **M. Yvonne Bobo,* Donnia L. Richardson, Loren D. Coen, and Victor G. Burrell,** S.C. Department of Natural Resources, Marine Resources Division, Charleston, SC 29412.

The oyster pathogen *Perkinsus marinus* is widely distributed in U.S. oyster populations from the Gulf of Mexico to Maine and is considered a major cause of mortality in oyster populations in the Gulf of Mexico and northeast. In South Carolina, over 95% of the oysters grow intertidally, with tides generally >1–2 m and elevated salinities and temperatures during exposure. Hence, disease epizootiology in the southeast may be very different from that observed for subtidal populations. Between 1972 and 1996, over 21,000 oysters from over 60 sites along South Carolina's coast were examined for the parasite. Gill, mantle and/or rectal tissues were dissected from 25 oysters from each site at each sampling time, and *Perkinsus marinus* infection diagnosed after incubation

in Ray's Fluid Thioglycollate Media. Each oyster was ranked using the Quick and Mackin scale of 0 (uninfected) to 6 (heavily infected), and weighted incidence (mean infection intensity) and prevalence (percent of the population infected) levels calculated for each sample. *Perkinsus marinus* was present in all of the populations examined since 1972. Prevalence and intensity of the disease varied with location, and generally the greatest levels occurred during late summer and early fall. However, whereas infections seem to disappear during the winter months in the northeast, in South Carolina they persist all year. Over this 24 year period, only 5% (or 42/831) of all composite oyster samples exceeded weighted incidence levels of 3.0. From 1972 to 1979, no mean intensities above 3.0 were observed. Then from 1980 to 1989, 71% (or 30 of 42) of all intensities exceeding 3.0 were observed, and finally, from 1990 to 1996, 29% (12 of 42) occurred. In South Carolina, *P. marinus* infections tended to more closely follow those observed in Gulf Coast oyster populations, rather than those typically observed in the northeast.

EFFECT OF LIPID SUPPLEMENTATION ON THE LIPID COMPOSITION AND GROWTH OF JUVENILE *TAPES PHILIPPINARUM* FED *TETRASELMIS SUECICA*. Marrit Caers,* Peter Coutteau, and Patrick Sorgeloos, Laboratory of Aquaculture and Artemia Reference Center, University of Ghent, Rozier 44, B-9000 Gent, Belgium.

Although information on bivalve nutrition is still limited, several studies indicated the importance of lipid quality and quantity of dietary lipids. The objectives of the present study were to investigate the (n-3) highly unsaturated fatty acid (HUFA) requirements, mainly 20:5n-3 (EPA) and 22:6n-3 (DHA), of juvenile clams and the effect of lipid supplementation on growth and the lipid and fatty acid composition of the seed. *Tetraselmis suecica*, which contained EPA but no DHA, was supplemented with a DHA rich emulsion. Daily growth rate, at 3 different algal feeding rations, whether or not supplemented with lipids, was compared with the growth rate of animals fed on a mixed algal diet which is known to support good growth. Lipid supplementation hardly improved the growth, suggesting that EPA could fulfill the (n-3) HUFA requirements of juvenile *T. philippinarum*, although a mixture of EPA and DHA may be slightly better. Animals fed lipid supplemented diets showed a significant increase of the total lipid content mainly as a result of an increase of the triacylglycerol content. Starved clams lost 26% of their initial lipid reserves. The fatty acid profile of *T. philippinarum* mainly reflected that of the diet. The supplementation of the emulsion resulted in a drastic increase of the DHA proportions in the animals while the EPA levels decreased. Starved clams maintained their initial DHA level, the EPA proportion decreased with 40%.

BIOCHEMICAL CHARACTERIZATION OF AN ENZY-MATIC CASCADE INVOLVED IN THE IMMUNE RE-SPONSE OF THE CRAYFISH *PROCAMBARUS CLARKII* TO NON-SELF MOLECULES. Washington Cardenas* and John R. Dankert, Department of Biology, University of South-western Louisiana, Lafayette, LA 70504.

Invertebrates are not known to contain a system of acquired or specific immunity. Host defense is non-specific and is mainly mediated by circulating hemocytes. In Arthropods, the pro-phenoloxidase system (proPO) is an enzymatic cascade system involved in the recognition of microbial molecules. This system resides in intracellular vesicles of the hemocytes. We have studied the biochemistry of the proPO activation in the crayfish *P. clarkii*. Extracted proPO from crayfish hemocytes was transformed to its active form "phenoloxidase" (PO) by a serine protease. This activation was affected by Ca^{++} concentration. Maximal spontaneous activation was observed at a Ca^{++} concentration of 5 mM. A component of fungal cell walls (β -1,3-glucan) was also effective in activating the pro-enzyme. Lipopolysaccharides (LPS) from Gram-negative bacteria activated the system after a lag time of 25 to 30 min. However, LPS derivatives (deacylated LPS, lipid A, and β -D-GlcNac-[1 \rightarrow 6]-D-GlcNac) were not able to activate the enzymatic cascade in *P. clarkii*. A serine protease has been shown to be involved in the activation of proPO by LPS. This suggests that the activation of proPO in *P. clarkii* might be mediated through the recognition of the "complete" LPS molecule by an endogenous serine protease.

ADVANTAGES OF USING A HELIOTHERMIC MARINE BASIN FOR ONGROWING HATCHERY REARED SCAL-LOP (*PECTEN MAXIMUS*) SPAT. Gyda Christophersen, Centre for Studies of Environment and Resources, University of Bergen, Bergen High-Technology Centre, N-5020 Bergen, Nor-way; Oivind Strand, Department of Aquaculture, Institute of Ma-rine Research, P.O. Box 1870 Nordnes, N-5024 Bergen, Norway.

The use of landlocked heliothermic marine basins in bivalve spat production has over a 100 year old history in Norway. The freshwater run-off to the basins forms a brackish surface layer of water which provides a "greenhouse effect" in the system. Studies of the carrying capacity in one of the landlocked basins has shown substantial possibilities for enhancement of phytoplankton and bi-valve spat production by manipulating the limiting factors. The water from this landlocked basin is now used as a food production system for a landbased raceway nursery where scallop spat are grown from 2 to 15 mm shell height. Transfer of hatchery reared spat during spring is desirable in order to extend the growth season for the small scallops. An early increase in temperature ($>10^{\circ}\text{C}$) can be controlled in the heliothermic basin. This makes the transfer of 2 mm scallop spat to the landbased nursery a more favourable solution than transferring spat to open sea conditions where a high mortality rate due to low sea-temperatures ($<7^{\circ}\text{C}$) in the spring has

been shown. The water taken from the basin is filtered to prevent fouling organisms and predators entering the raceways. This growth system has a low demand of labour compared to growth systems in the sea. Survival in groups of up to 0.5 million spat transferred from the hatchery to the nursery during April to August, were 25–70%. Growth of the spat is similar to the highest growth rates obtained in sea conditions during summer. Studies are now in progress to increase the knowledge of environmental factors in the basin influencing scallop spat production.

EFFECT OF PLANTING DENSITY AND PREDATOR EXCLUSION ON GROWTH AND SURVIVAL OF NORTHERN HARD CLAM *MERCENARIA MERCENARIA* AT THE INDIAN RIVER LAGOON, FLORIDA. Eva M. Fernandez* and Junda Lin, Florida Institute of Technology, Dept. of Biol. Sci., Melbourne, FL 32901; John Scarpa, Harbor Branch Ocean. Inst., Fort Pierce, FL 34951.

Growth and survival of the northern hard clam *Mercenaria mercenaria* cultured in nylon mesh bags (1.20 × 1.20 m) was assessed against stocking density, seed size, and presence or absence of a predator exclusion device (Vexar net with 2.5 cm openings) in the Indian River Lagoon at Oak Hill, Florida. Each density was replicated four times for both protected and unprotected treatments. Bags were sampled monthly to determine growth and survival. Nursery seed (6–6.5 mm shell length, SL) were stocked in 3 mm mesh size bags at densities of 7,500, 10,000 and 12,500 clams/bag. Growout seed (20–22 mm SL) were stocked in 10.5 mm mesh size bags at the densities of 750, 1,000 and 1,250 clams/bag. The first monthly sampling of growout seed showed SL to be different among treatments: protected 2.55 ± 1.4 mm, 2.03 ± 1.9 mm, and 1.8 ± 1.7 mm; unprotected: 2.1 ± 1.8 mm, 1.4 ± 1.7 mm, and 2.51 ± 1.3 (750, 1,000 and 1,250 clams/bag, respectively) with no indication of differential survival. Growth and survival will be correlated with temperature, salinity and turbidity measurements.

A MATHEMATICAL MODEL FOR *HAPLOSPORIDIUM NELSONI* (MSX)-OYSTER INTERACTIONS. Susan Ford* and Eric Powell, Haskin Shellfish Research Laboratory, Rutgers University, Port Norris, NJ 08345; Eileen Hofmann and John Klinck, CCPO, Old Dominion University, Norfolk, VA 23529.

Mathematical models are valuable for integrating a wide variety of biological data into useful management tools. A model simulating the host-parasite-environment interactions of eastern oysters, *Crassostrea virginica*, and the pathogen *Perkinsus marinus* (Dermo) is already available. We now have constructed a second model describing the relationship between eastern oysters and another pathogen, *Haplosporidium nelsoni* (MSX). Steps in model building have included converting the infection intensity staging system into parasites per gram wet weight in epithelial and

subepithelial tissues; determining lethal parasite densities; and constructing biological rate equations for parasite growth (including sporulation) and death rates under various ambient conditions based on field observations and experimental data. In the model, host-parasite interactions are governed by ambient environmental conditions of temperature, salinity, food availability, and turbidity, as well as by density-dependent biological controls on the parasite. Simulations that use environmental conditions characteristic of Delaware Bay reproduce the observed seasonal *H. nelsoni* cycles and consequent oyster mortality in oysters with varying degrees of resistance to the parasite. In model simulations, the amount of cold exposure in winter, the timing of the spring plankton bloom, and summer salinity are the primary factors controlling *H. nelsoni* prevalence and intensity.

Our next step will be to insert the *H. nelsoni* model into the existing *P. marinus*-oyster model. The resulting dual parasite model, describing the effects of both *P. marinus* and *H. nelsoni* on oysters, will provide a valuable synthesis of interactions between environment, parasites, and populations of eastern oysters, which are frequently affected by both pathogens. The resulting model can be used to guide future laboratory and field studies as well as management efforts.

OYSTER SPATFALL MONITORING IN SOUTH CAROLINA USING FRENCH COLLECTOR TUBES. Nancy H. Hadley,* M. Yvonne Bobo, A. J. Erskine, and L. D. Coen, SC Dept of Natural Resources, Marine Resources Research Institute, Charleston, SC 29412.

In 1996, as a part of a larger study to determine status of South Carolina oyster resources, we conducted a spatfall study at 23 sites along the South Carolina coast. "French collector" sticks (~1.8 cm OD, 1.0 m height) were deployed in early August and retrieved in late October. Five collectors were deployed at each site by pushing the collector into the substrate to a depth of 45 cm, leaving 55 cm exposed. All collectors were placed at approximately the same tidal height, parallel to shore, with an interval of 5–10 meters between collectors. The collectors were deployed at a target height of 0–0.3 m above mean low water. Of the 115 collectors deployed, 111 (96%) were retrieved. After retrieval, collectors were maintained in an upright position in a refrigerated room for 1–10 days prior to enumeration. For enumeration, collectors were marked at 15 cm intervals from the substrate line and spat were enumerated within each interval using a magnifying loop, allowing recognition of spat as small as 2 mm. Surface area of exposure was calculated by multiplying the exposed length by a calculated circumference of 11.25 cm, allowing counts to be expressed as spat/cm².

Mean spat density at the 23 sites ranged from 0–0.28 spat/cm², with a grand mean of 0.096 spat/cm². Only one site recorded no spatfall. Within sites, collectors were generally similar, but there were marked differences between sites, even when separated by

less than 2 km within the same creek system. Site differences may result from a number of factors, including health of surrounding oyster populations, deployment timing relative to spawning events, water current patterns, presence of predators, and water quality. Results also differ from previous and even concurrent spatfall studies which used different methodologies. Interpretations of spatfall data must take into account factors such as type of collector, tidal height, time and length of exposure.

CHARACTERIZATION OF THE ACTIVATION OF THE PHENOLOXIDASE SYSTEM OF HOST DEFENSE IN THE OYSTER *CRASSOSTREA VIRGINICA*. Percy J. Jordan,* Lewis E. Deaton, Washington Cardenas, and John R. Dankert, Department of Biology, University of Southwestern Louisiana, Lafayette, LA 70504.

The response of oysters to pathogenic organisms may involve the activation of an enzyme that is present in an inactive form known as prophenoloxidase (proPO). This enzyme has been detected in the hemolymph of a wide variety of invertebrates, and is usually localized in circulating hemocytes. The activation of proPO to phenoloxidase has been associated with host defense in arthropods. The activity of phenoloxidase (PO) can be detected and quantified spectrophotometrically by oxidation of the substrate L-3,4-dihydroxyphenylalanine. We assayed hemolymph from *Crassostrea virginica* for PO and found that significant enzyme activity is located both free in the plasma and in the hemocytes. The PO activity in the plasma is increased by the addition of an exogenous serine-protease (trypsin), and by molecules of microbial origin such as Zymosan A and bacterial lipopolysaccharides. These results suggest that the activation of proPO in the plasma may involve a proteolytic cascade similar to that involved in the clotting of vertebrate blood, and that the PO system in oysters is activated by foreign proteins. We speculate that the PO system may serve to label foreign pathogens as targets for phagocytosis by hemocytes. Supported by the Whitehall Foundation and the Louisiana Educational Quality Support Fund.

INITIAL CHARACTERIZATION OF THE HEMOLYMPH PHENOLOXIDASE SYSTEM IN THE SCALLOPS *ARGOPECTEN IRRADIANS* AND *PLACOPECTEN MAGELLANICUS*. Percy J. Jordan,* Lewis E. Deaton, Washington Cardenas, and John R. Dankert, Department of Biology, University of Southwestern Louisiana, Lafayette, LA 70504.

The response of molluscs to pathogenic organisms may involve the activation of an enzyme that is present in an inactive form known as prophenoloxidase (proPO). This enzyme has been detected in the hemolymph of a wide variety of invertebrates, and is usually localized in circulating hemocytes. The activation of proPO to phenoloxidase has been associated with host defense in

arthropods. The activity of phenoloxidase (PO) can be detected and quantified spectrophotometrically by oxidation of the substrate L-3,4-dihydroxyphenylalanine. We assayed hemolymph from bay and rock scallops for PO and found that significant enzyme activity is located both free in the plasma and in the hemocytes. The PO activity in the plasma is increased by the addition of an exogenous serine-protease (trypsin), and by molecules of microbial origin such as Zymosan A and bacterial lipopolysaccharides. These results suggest that the activation of proPO in the plasma may involve a proteolytic cascade similar to that involved in the clotting of vertebrate blood, and that the PO system in molluscan hemolymph is activated by foreign proteins. We speculate that the PO system may serve to label foreign pathogens as targets for phagocytosis by hemocytes. Supported by the Whitehall Foundation and the Louisiana Educational Quality Support Fund.

SEA SCALLOP ENHANCEMENT AND CULTURE IN NEW ENGLAND. Richard Langan,* University of New Hampshire, Jackson Estuarine Laboratory, 85 Adams Point Road, Durham, NH 03824; Sue Kuenstner, New England Fisheries Development Association, 451 D Street, Boston, MA 02210; G. Jay Parsons, Marine Institute of Memorial University, St John's, NF, A1C 5R3, Canada; Sandra E. Shumway, Natural Science Division, Southampton College, L.I.U., Southampton, NY 11968; Mark Simonitsch, Fish Weirs, Inc., 84 Doane Road, Chatham, MA 02633.

In early 1996, a sea scallop (*Placopecten magellanicus*) aquaculture project was begun to investigate spat collection and to evaluate growth of juveniles in various areas of New England. The study sites have been established, and analysis of preliminary results will begin in the spring of 1997. Spat collection efforts are ongoing at both coastal and offshore sites. Enhancement of local scallop populations through spat collecting activities will be investigated. Spat removed from collector bags after three months and held in upwellers will be compared to those which overwinter on the collectors, to determine if larger and hardier seed scallops can be produced by maintaining spat in a "nursery." An on-board spat sorting system will be developed as a means of decreasing handling and mortality of animals. Growth rates of juvenile scallops held in pearl nets and benthic cages will be determined at three sites. In the event that toxin-free scallops are produced, whole and/or roe-on scallops will be test marketed.

DEVELOPMENT OF ANESTHETICS FOR THE MUSSEL *ELLIPTIO COMPLANATA*. William A. Lellis* and Timothy A. Pierhophles, United States Geological Survey, Biological Resources Division, Research and Development Laboratory, Wellsboro, PA 16901.

An experiment was conducted to develop a safe and reliable method of anesthetizing the freshwater mussel *Elliptio complanata*

for collection of biological samples and assessment of reproductive status. Various combinations and concentrations of MS-222, magnesium chloride, potassium chloride, 2-phenoxyethanol, and succinylcholine chloride were administered by bath, slow drip, or injection into the gills or foot. Mussels were considered anesthetized when the valves opened approximately 1 to 2 cm and the animal became impervious to touch. MS-222 administered at concentrations of 75 to 250 ppm produced an opening of the valves and extension of the foot, but the mussels quickly retracted when disturbed. Phenoxyethanol at concentrations of 0.25 to 3.0% produced anesthesia of 5 to 70% of the mussels within 2.5 to 4.0 hours after administration. Anesthesia rate improved to 90% when mussels were first relaxed with 100 ppm MS-222. Injection of 0.5 to 5.0 mg succinylcholine chloride into the foot of mussels previously relaxed with 100 ppm MS-222 produced rapid anesthesia which lasted from 20 to 40 minutes. Neither magnesium chloride nor potassium chloride produced anesthesia in *Elliptio complanata*. Data indicate that succinylcholine chloride produced a quick, short-term anesthesia in *Elliptio complanata*, whereas 2-phenoxyethanol produced a slower, but longer-lasting effect.

DISTRIBUTION AND COLOR MORPH ECOLOGY OF GREEN CRABS IN SOUTHERN NEW ENGLAND. Aly McKnight,* Department of Forestry and Wildlife Management, University of Massachusetts, Amherst, MA 01002; Lauren Mathews, Department of Biology, University of Southwestern Louisiana, Lafayette, LA 70535, Rachel Avery, Box 3043 Connecticut College, New London, CT 06320; Karen T. Lee, Department of Biology, University of Pittsburgh, Johnstown, Johnstown, PA 15904.

The invasive marine crab *Carcinus maenas* colonized eastern North America in the mid-nineteenth century. Since its introduction, *C. maenas* has become a prominent species in southern New England, and poses a threat to commercial mollusk fisheries along the New England coast. In Europe *C. maenas* exhibits a range of coloration from pale green through red. Physiological and distributional differences between green and red morphs have been documented. This study attempted to discover whether western Atlantic populations of *C. maenas* exhibited a similar color morph ecology. Crabs were collected from sites in New England. Distribution of crabs was evaluated in subtidal, intertidal and estuarine sites, and morphological characteristics were recorded for all captured crabs. Green morphs outnumbered red morphs in all habitat types; however, red morphs were more abundant in subtidal sites than in any other habitat type. In estuarine sites, red morphs became less abundant with increasing distance from the estuary mouth. Red morphs were larger on average than green morphs. In subtidal crabs, red morphs exhibited more carapace fouling than green morphs, and red males were found to suffer a greater incidence of limb loss than green males. These results generally followed trends reported in European populations.

FEASIBILITY OF USING DETRITAL BASED DIETS TO SUPPORT GROWTH AND SURVIVAL OF NATIVE CLAMS. S. Jerrine Nichols,* Great Lakes Science Center, USGS, 1451 Green Rd., Ann Arbor, MI 48105.

Native clam survival in captivity is often limited due to the difficulties in providing food. We are presently having success in using detrital-base diets to support consistent growth of young native clams of several species in the laboratory. The base used to form these diets appears to be immaterial if the following conditions are met: (1) the food material must be complete, containing all the essential amino acids, and fatty acids such as linoleic or linolenic. Incomplete forage base either leads to no growth or death; (2) the diet must be pre-digested or rotted before offering it to the clam—fresh diets may be digested, but have limited assimilation, and lead to very inconsistent growth and survival rates; (3) food must be supplied at a rate of at least 4mg/L for at least 12 hours a day, preferably longer; (4) water quality must somehow be maintained. Basically, these diets are actually infusoria cultures, replete with various types of bacteria. Our greatest challenge remains in maintaining water quality. We are presently managing 8000 clams in the laboratory on these diets. Survival of older individuals is excellent, and younger clams are increasing in shell length. The next few months will be critical in determining whether this kind of forage base remains a feasible option in meeting the dietary needs of native clams.

ASSESSMENT OF BIOLOGICAL PARAMETERS FOR MANAGEMENT OF MUSSEL SEED COLLECTION IN AMHERST BASIN (MAGDALEN ISLANDS, QUÉBEC). Marcel Roussy* and Bruno Myrand, MAPAQ, Direction de l'Innovation et des Technologies, Station Technologique Maricole des Îles-de-la-Madeleine, C.P. 658, Cap-aux-Meules, Québec, Canada G0B 1B0.

Amherst basin is the only seed collection site used by the mussel growers from Magdalen Islands. Therefore, information is needed in order to assure appropriate management of this site. The reproduction, the larval cycle and the timing and intensity of spat-fall were followed in 1995 and 1996. There were three spawning events in 1995 and only two in 1996. The spawnings of June 10, 1995 and June 8, 1996 supplied the bulk of spat which settled down on collectors. There were only two spatfall peaks in 1995, the first in late June and the second in early August, and only one in 1996 in late June. Collectors provided the same amount of spat (4500 ind/m) both years despite a much greater larval density in 1995. The collectors immersed before July 10, 1995 and June 25, 1996 gave the maximum yield. Spat showed two modes in shell length (8 and 17 mm) in mid-September both years. Only one of the two mussel populations studied contributed clearly to the first spawning event of 1996 despite the proximity (900 m) of the beds. This arises interrogations on the usefulness of a methodology

based on the evolution of dry tissue mass of wild adults to identify spawnings responsible for spat collection.

ASPECTS OF THE LIFE CYCLE OF *HEMATODINIUM PEREZI* IN THE BLUE CRAB, *CALLINECTES SAPIDUS*.

Jeffrey D. Shields,* Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062; **Gretchen A. Messick,** NOAA, National Marine Fisheries Service, Northeast Fisheries Science Center, Oxford, MD 21654.

Little is known of the life cycle of *Hematodinium perezii*. Recent culture of the parasite has helped to study its life cycle *in vitro*, but certain aspects remain unknown. In the blue crab, the parasite occurs in the hemolymph where it is found in four different stages. The motile plasmodium is found in early infections, and probably arises from an infectious dinospore. The trophont occurs in at least two morphologically different stages: an amoeboid form with few, small refractile granules, and a large rounded form with many, large refractile granules. The latter may represent a sporont as it is generally observed in later stages of infection. Dinospores are rarely observed in the hemolymph, but they are quite common in culture. Dinospores of *H. perezii*, like other syndinids, occur as either microspores or macrospores. The role of the different spore types is unknown but an infection appears to produce only one spore type. In aquaria, crabs with light infections (1 plasmodium/100 host cells) develop heavy infections (>100 trophonts/100 host cells) over two to three weeks. In fatal infections few host cells remain, and small, rounded forms of the parasite (prespore or effete stage) are often observed in the hemolymph. Since cultures are now established, we plan to investigate the role of the dinospore in the transmission of the disease.

MOLECULAR DETERMINATION OF SYMBIONT TRANSMISSION STRATEGIES IN WOOD-BORING BI-VALVES (FM, TEREDINIDAE).

Alison R. Sipe,* Ami E. Wilbur, and **S. Craig Cary,** University of Delaware-College of Marine Studies, Lewes, DE 19958.

Teredinid shipworms are associated with cellulolytic nitrogen-fixing bacterial endosymbionts enabling them to degrade wooden marine structures and cause millions of dollars of damage globally each year. The mechanism by which shipworms acquire these symbionts has been investigated by localization of the symbionts in host tissue using molecular hybridization methodologies. We screened adult gonadal tissue and larvae in different stages of development for the presence of the symbiont using the polymerase chain reaction (PCR) with primers specific for the gene encoding the bacterial small sub-unit ribosomal RNA (16S rRNA). Positive amplifications were verified by restriction fragment length polymorphism (RFLP) and sequence analysis, and the endosymbiotic nature of the bacteria confirmed by *in situ* hybridization. Results from two teredinids (*Bankia setacea*, collected from Yaquina Bay, OR, and *Teredo navalis*, collected from Delaware

Bay, DE) indicate that the bacterial symbionts are acquired from the parents, which contradicts the expectation of environmental acquisition suggested by previous work. Additionally, symbiont 16S rRNA phylogenetic analysis supports the notion that host species each harbor a unique bacterial symbiont. Further understanding of the intricacies of this symbiotic association may provide insight into the mechanisms involved in shipworm larval recruitment and how these processes may be disrupted to prevent degradation of marine timber.

VARIATION IN EMERSION AND THERMAL TOLERANCES OF SELECTED FRESHWATER UNIONID MUSSELS.

Diane L. Waller,* W. Gregory Cope, Michelle R. Bartsch, and **James A. Luoma,** U.S. Geological Survey, Upper Mississippi Science Center, P.O. Box 818, La Crosse, WI 54602.

The success of conservation activities designed to protect unionid mussels, such as relocations and status surveys, depends on the mussel's ability to survive handling and to re-establish in the substrate after displacement. We evaluated the effects of handling and emersion on unionid mussels at extreme water and air temperatures that are likely to be encountered during survey and relocation activities. Five laboratory tests were performed with *Lampsilis cardium* (n = 2), *Quadrula pustulosa* (n = 1), and *Elliptio dilatata* (n = 2) mussels. Each test was conducted in a randomized design as a factorial experiment with the main effects confounded. Each species (except *Q. pustulosa*) was tested at two water temperatures (10 and 25°C), each at five air temperatures (ranging within $\pm 20^\circ\text{C}$ of the water temperature), and three emersion intervals. All treatments were duplicated, with 10 mussels per emersion time and air temperature (n = 320 mussels/test). Mortality and behavioral responses (orientation and burrowing) were measured daily for 14 d post-exposure. Results showed that only *E. dilatata* experienced significant treatment related mortality at the 25°C water temperature after extended emersion at high air temperatures. Behavioral responses were directly correlated with water temperatures.

CHANGES IN ASPECTS OF THE BLOOD CHEMISTRY OF BLUE CRABS INFECTED WITH *HEMATODINIUM PEREZI*.

Diana M. Whittington,* Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA, 23062; **Kyrie L. Bernstein,** Department of Biology, University of Virginia, Charlottesville, VA 22904; **Jeffrey D. Shields,** Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA 23062.

The parasitic dinoflagellate *Hematodinium perezii* infects the hemolymph of the blue crab *Callinectes sapidus*. Infected crabs frequently show signs of weakness and lethargy, and often die to stress-related handling or fishing. Radical changes in the chemistry of the hemolymph are obvious by the lack of clotting ability, and by its frequently observed discoloration. We investigated some basic elements of the hemolymph as an initial characterization of

pathological changes that occur in infected crabs. Total protein levels were significantly different between infected and uninfected male crabs, but not between similar female crabs. Acid phosphatase activity in the hemolymph varied significantly between maturity status of the hosts, but significant differences between diseased and uninfected status were only observed in male crabs. Acid phosphatase levels in the hemolymph of infected male crabs were an order of magnitude higher than that observed in uninfected males. Levels in female crabs were not significantly different. Lipid classes were also analyzed and significant differences were observed between uninfected and infected hosts. Differences between sexes are being investigated, but evidence suggests that the infection takes longer to fulminate in females than males.

A NUCLEAR MARKER FOR THE MOLECULAR IDENTIFICATION OF KUMAMOTO OYSTER (*CRASSOSTREA SIKAMEA*) BROODSTOCK. Ami E. Wilbur* and Patrick M. Gaffney, College of Marine Studies, University of Delaware, Lewes, DE 19958.

In recent years, commercial culture of Kumamoto oysters (*Crassostrea sikamea*) has been hindered by the presence of non-

Kumamoto like offspring which complicate the rearing process and reduce crop value. These contaminants are due to inclusion of *C. gigas*, and their hybrids (*sikamea* ♀ × *gigas* ♂) in hatchery broodstock. Additionally, the apparent near-extinction of *C. sikamea* in its native Japanese waters has prompted a search for remnant Kumamotos surviving from introductions in the Pacific Northwest in the 1950's. The inability to reliably identify Kumamoto oysters using morphological traits has generated interest in the development of a diagnostic assay for this species. Pure *C. gigas* individuals can be identified using mitochondrial markers, but currently, identification of hybrids is indirect and involves allozyme analysis of juveniles produced in controlled crosses. We have developed a diagnostic nuclear marker based on PCR/RFLP techniques that allows direct testing of potential broodstock. Restriction digest of the ITS-1 PCR product (a non-coding nuclear region between the 18S and 5S rRNA genes) distinguishes between *C. gigas*, *C. sikamea* and the native Pacific Northwest oyster, *Ostrea conchaphila*. To date we have typed 300 putative Kumamotos in an ongoing effort to help preserve the integrity of this species and to identify pure Kumamoto broodstock for the culture industry.

ERRATUM

Kleinschuster, S. J., J. Parent, C. W. Walker & C. A. Farley. A cardiac cell line from *Mya arenaria* (Linnaeus, 1759). *J. Shellfish Res.* 15(3):695–707.

Page 706: Sentence should read: On the basis of our observation of reduced mitotic activity after the first passage of the cultures, we expect that the generation number of the cardiac cells therein will be finite, as are all normal cells.

The printer regrets the error.

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COVER PHOTO: Veliger of queen conch, *Strombus gigas*, approximately 21 days old, ready for settlement. (Photo by Megan Davis)

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EFFECT OF EYESTALK ABLATION ON MOLT CYCLE AND REPRODUCTION IN THE BANDED CORAL SHRIMP, *STENOPUS HISPIDUS* (OLIVER)

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ABSTRACT This study examined the effect of eyestalk ablation on molt cycle and reproduction of the banded coral shrimp, *Stenopus hispidus* (Oliver), a decapod popular among marine aquaria hobbyists. Eyestalk ablation significantly shortened the molt interval in the male shrimps: 11–15 (12.3 ± 0.7 , mean \pm SE, $n = 6$) days compared with 15–27 (21.3 ± 0.9 , mean \pm SE, $n = 6$) days for the nonablated (control) individuals. The effect decreased gradually. By the third molt, the interval was not significantly different between the ablated and intact shrimps. The result was more complicated for the female shrimps. The females were divided into two groups: “stagnant” shrimps (ovary remained undeveloped for a long time) and ovigerous shrimps with eggs artificially removed. In the “stagnant” shrimps, eyestalk ablation significantly enhanced gonadal development. The ovaries developed (became green) on the fourth day after the ablation and spawned on the eighth day (8.3 ± 0.3 days, mean \pm SE, $n = 4$). In contrast, nonablated individuals spawned in 18–25 (22.5 ± 1.5 , mean \pm SE, $n = 5$) days. Once spawned, there was no significant difference in ovarian development between the ablated and control shrimps. In the shrimps with eggs removed, neither unilateral nor bilateral eyestalk ablation stimulated ovarian development and maturation. The unilaterally ablated, bilaterally ablated, and intact females spawned in 15.5 ± 0.3 , 15.4 ± 0.3 , and 15.8 ± 1.1 (mean \pm SE, $n = 5$) days, respectively. This study suggests that eyestalk ablation can be used to promote the ovary redevelopment in the banded coral shrimp.

KEY WORDS: banded coral shrimp, *Stenopus hispidus*, eyestalk ablation, molt, reproduction

INTRODUCTION

Since Zeleny's study (1905), in which he removed both stalked eyes of the fiddler crab (*Uca pugilator*) and observed a decrease in the molt interval, this simple procedure of eyestalk ablation has been used in many crustacean species, especially decapods (see a review by Chang 1989). With increasing knowledge of the endocrine activity and its control of gonad development in crustaceans, the technique is receiving greater attention as a method of inducing precocious maturation of the ovary and subsequent spawning in captivity (Browdy and Samocha 1985, Primavera 1985 for a review, Makinouchi and Primavera 1987).

Crustacean eyestalks are known to contain the neuroendocrine center, X-organ-sinus gland complex, that is responsible for storing and releasing both the molt- and gonad-inhibiting hormones (MIH, GIH). MIH has been found to inhibit the secretion of molting hormones (MH) by the Y-organs (Soumoff and O'Connor 1982, Mattson and Spaziani 1985). It is long known that molting and gonadal development in crustaceans are regulated in a complicated way by the hormones: MIH, GIH, MH, GH (gonad hormone), and JHs (juvenile hormones). The mechanisms through which these hormones control molting and gonad development have been investigated (see reviews by Passano 1960, Adiyodi and Adiyodi 1970, Cooke and Sullivan 1982, Chang 1984, Chang 1985, Kleinholz 1984, Fingerman 1987, Laufer and Landau 1991). The MH ecdysteroid is a crucial hormone that influences molting and reproduction (see Chang 1989 for a review). The concentration of MH varies during the course of the molt cycle in a number of different crustacean species (reviewed in Chang 1989). Immediately after egg extrusion, the circulating

level of ecdysteroids in the female crab (*Cancer anthonyi*) is low. The concentration then steadily increases to a maximum just before hatch. Immediately after hatch, the female undergoes ovarian development without an intervening molt. As the ovaries develop, circulating levels of ecdysteroids decrease to a minimum just before subsequent egg extrusion (Chang 1991). Similar results were obtained in the shrimp, *Palaemon serratus* (Spindler et al. 1987). There is a significant delay of molt due to egg extrusion in American lobster, *Homarus americanus* (Chang 1984). Brood incubation results in longer molt cycle in the isopod (*Oniscus asellus*) (Steel 1980), and no females with developed ovaries were molting in *Penaeus canalicularis* (Choy 1987). Molting crabs (*Carcinus maenas* and *Paratelphusa hydromous*) with developing ovaries have never been observed in nature (Adiyodi and Adiyodi 1970 for a review). These studies indicate that crustaceans can coordinate molt and reproduction.

Our study assessed the effect of eyestalk ablation on the ovarian development and the molt cycle of the banded coral shrimp, *Stenopus hispidus*. The banded coral shrimp is a popular aquarium species. Its low abundance and delicate nature make its collection challenging. Efforts have been made to develop the larval rearing method for the shrimp (e.g., Young 1979, De Castro and Jory 1983, Fletcher et al. 1995). Generally, a mature female (>3.5 cm in total length) has a molt/reproduction cycle interval of about 16 days at temperatures of 26–30°C. Immediately after hatch, the female molts, mates with a male, and undergoes ovarian development without an intervening molt (Zhang et al. in press). However, ovaries of females collected from the wild or purchased from a pet store are usually undeveloped (“stagnant”). It takes a long time

(about 30 days) for the ovary to redevelop. Shortening this duration will benefit the production of the shrimps.

Removal of the eyestalk results in precocious gonadal development and decrease in molt interval, but the effect is different among the life stages. Chang (1989) found that eyestalk ablation cannot shorten molt cycle and promote gonadal development in lobsters once the female enters the sexual maturation cycle. It is not known whether this is a pervasive phenomenon in crustaceans. In the isopod (*O. asellus*), appearance of premolt is greatly delayed if one or two eggs remained stuck in the pouch (Steel 1980).

In this study, we tested the response of the banded coral shrimp (in both males and females) to eyestalk ablation. We tested the hypothesis that the effect of eyestalk ablation is different for animals of different reproduction stages, specifically before and after ovarian development. We also investigated whether the embryo and gonad affect the molting cycle regulation.

MATERIALS AND METHODS

The shrimps (total length: 3.0–4.2 cm for male and 4.0–4.8 cm for female) were purchased from local pet shops that had them collected from the wild (1–2 days before our purchase). They were maintained in a recirculating seawater system under 14 h light: 10 h dark. Each pair (one female and one male) was cultured in a 25-L plastic tank in a greenhouse. Water temperature was kept at 26–31°C. The shrimps were acclimated for a week before the study and fed in excess with frozen *Artemia* or squid once a day.

For each experimental shrimp, the eyestalk was ablated at the base with forceps. The same number of nonablated shrimps was used as controls. Eyestalks of six males were unilaterally ablated 20 h after molting. The first three molt cycle intervals after the ablation were recorded. The females in two states, "stagnant" and with developing gonad, were used. Four "stagnant" females were used to test the effect of unilateral eyestalk ablation on ovarian development. The first two molt cycle intervals after the ablation were recorded. Five females with developing gonads were used for unilateral and bilateral eyestalk ablation, respectively. This experiment was to test whether removal of the X-organ through eyestalk ablation is effective in shortening the molt cycle and promoting gonad development of the shrimps during the reproductive period.

The embryos were removed with forceps on the day of egg extrusion, and the eyestalks were ablated 20 h later. Because the oviposition occurs just after the ovigerous females molt and mate, molt cycle can be used to indicate the ovarian development cycle. Student's *t*-test was used to compare molt interval between ablated and intact shrimps.

RESULTS

Male

For the males, the molt interval after eyestalk ablation was 11–15 days (12.3 ± 0.7 , mean \pm SE) compared with 15–27 days (21.3 ± 0.9 , mean \pm SE) for nonablated individuals ($p < 0.001$, Table 1). The difference decreased gradually over time and became not significant by the third molt ($p > 0.05$, Table 1).

Female

Eyestalk ablation significantly shortened the molt cycle of "stagnant" females ($p < 0.001$, Table 1). The ovaries of ablated "stagnant" females developed (ovary became green) on the fourth day after the ablation, and spawning/molt occurred on the eighth day (8.3 ± 0.3 , mean \pm SE). In contrast, spawning of intact individuals occurred in 18–25 days (22.5 ± 1.5 , mean \pm SE). The second molt cycle interval of ablated "stagnant" females was 15.3 ± 0.3 days (mean \pm SE), not significantly different ($p > 0.05$) from that of the intact individuals (15.4 ± 0.3 days, mean \pm SE). For ovigerous females with eggs (embryos) removed, eyestalk ablation (both unilateral and bilateral) was not effective in shortening the molt cycle interval (Table 1), or in promoting gonadal development.

DISCUSSION

The most successful and common application of eyestalk ablation is in *Penaeus* shrimp broodstock culture (Browdy and Samocha 1985, Primavera 1985 for a review). This study shows that eyestalk ablation can also promote both molting and gonadal development of the banded coral shrimp. Unilateral eyestalk ablation significantly and substantially reduces the molt interval in the "stagnant" females. However, once a female enters the gonadal developing period, eyestalk ablation has little effect on the

TABLE 1.

The effect of eyestalk ablation on molt interval (day) in the banded coral shrimp, *S. hispidus* (mean \pm SE).

Gender		Molt After Ablation		
		1st	2nd	3rd
Male	UESA	$12.3 \pm 0.7^*$	$16.5 \pm 1.1^\dagger$	19.8 ± 2.1
(n = 6)	Intact	21.3 ± 0.9	18.8 ± 1.7	18.2 ± 1.3
Female I	UESA	$8.3 \pm 0.3^*$	15.3 ± 0.3	
(n = 4)	Intact	22.5 ± 1.5	15.4 ± 0.3	
Female II	UESA	15.5 ± 0.3	15.8 ± 0.5	
(n = 5)	BESA	15.4 ± 0.3	15.4 ± 0.3	
	Intact	15.8 ± 1.1	15.4 ± 0.4	

UESA, unilateral eyestalk ablation; BESA, bilateral eyestalk ablation; Female I, "stagnant" (gonad undeveloped); Female II, ovigerous female, with the eggs (embryos) removed.

* $p < 0.001$.

† $p < 0.05$.

molt cycle and gonadal development. For the female shrimps with their embryos removed, there was no significant difference in molt cycle interval between ablated and control individuals. The results show that the banded coral shrimp has an ability to coordinate molting and reproduction (possibly by regulating secretion of hormones) during the reproductive period. Because the ablated eyestalk cannot be regenerated, the hormones produced by the Y-organs would be expected to remain uninhibited, and the molt cycle would remain shorter. The results in this study do not support these findings. Similarly, after the fourth molt, the molt interval of the ablated American lobster is not significantly different from that of the intact controls (Chang 1989). In the isopod (*O. asellus*), the molt cycle of females is influenced by reproduction. Molt cycles of brood females are apparently longer than those of nonbreeding animals (Steel 1980). Obviously, this coordination is to allow sufficient time for embryo development. Endocrinology studies also support this. Eastman-Reks and Fingerman (1984) found that ovarian inhibiting hormone (OIH) secreted by the X-organ has no effect on the ovaries already begun in yolk accumulation. This means that eyestalk ablation does not stimulate ovarian development once the maturation process has been triggered. In this study, the results showed that whether or not the X-organ is intact in females with developed ovary, the molt cycle remained unchanged. There are two possible explanations for this: the activity of the X-organ is lower during the reproduction cycle (Han and Kim 1993) or MIH has the same action mechanism as OIH.

Even if eyestalk ablation does not affect the molt and reproduction cycle of the females with developed gonad or embryos, it

is still possible that gonad and embryo play a role in the regulation of the molt cycle. Steel (1980) found that one or two eggs remained stuck in the pouch can greatly delay the appearance of premolt in the isopod (*O. asellus*). Chang (1984) hypothesized that embryos communicate their presence to the mother's central nervous system (CNS) to inhibit molting. There is no evidence to support this hypothesis, however. The results presented here showed that embryos do not seem to affect the CNS. Gonads produce ecdysteroids in several crustacean species (Quackenbush 1986) and may play an important role in the complex regulation. Y-organ, a major source of MH, certainly plays a critical role. Histological studies in a crab *Hemigrapsus* sp. have shown that activity of two kinds of neurosecretory cell, A'- and B'-cell in the thoracic ganglion, are related to both X-organ and Y-organ (Matsumoto 1962). There was a different response to eyestalk ablation between mature males and females. The effect of eyestalk ablation in males decreased gradually. The cause of this difference is unknown.

The coordination between molt and reproduction is a complex process in crustaceans. Future work should focus on the change in MIH titer in the hemolymph, the action on target tissues, and histological changes in the X-organ during the molt period. On a more practical aspect, research should be done to examine whether eyestalk ablation affects fecundity and egg quality.

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LARVICULTURE AND EFFECT OF FOOD ON LARVAL SURVIVAL AND DEVELOPMENT IN GOLDEN CORAL SHRIMP *STENOPUS SCUTELLATUS*

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ABSTRACT The golden coral shrimp *Stenopus scutellatus*, a popular species in aquarium industry, was cultured in the laboratory. Three kinds of foods, *Artemia* nauplii, rotifer, and microalga *Chaetoceros gracilis* (Cg), were used to examine their effects on the larval survival and development. The larvae showed higher survivorship and survived longer when fed with *Artemia* nauplii than those fed with rotifer and Cg. All larvae fed with rotifer and Cg died on Days 12 and 11, respectively. No difference in development rate between larvae fed with *Artemia* nauplii and rotifer was observed. Larvae fed with Cg developed slower and could not develop beyond zoea II stage. The larvae were successfully reared to postlarval stage, indicating that the golden coral shrimp is a promising candidate for commercial aquaculture.

KEY WORDS: golden coral shrimp, *Stenopus scutellatus*, larviculture, food

INTRODUCTION

Golden coral shrimp, *Stenopus scutellatus*, distributes from Bermuda to Fernando de Noronha, Brazil, from shallow water to 55 m water depth (Holthuis 1946). The shrimp lives on isolated solid substrates in or near large beds of turtle grass (*Thalassia*) in protected, quite waters (Limbaugh et al. 1961). It is one of the most popular species in the aquarium industry because of its striking color: the body and leg are lemon yellow with bands of red.

The golden coral shrimp is a member of cleaning shrimps living in coral reef systems. The abundance of cleaning shrimp is low in natural environment, and removal of them results in reduction of reef fish abundance and a high incidence of fishes with frayed fins and ulcerated sores (Limbaugh et al. 1961, Glynn 1983). Extensive and destructive collection of coral reef organisms has caused increasing concern among conservationists and environmental biologists. Efforts have been made to reduce the gap between demand and supply through aquaculture.

Among the eight species in the genus *Stenopus* (Gurney 1936), the banded coral shrimp, *Stenopus hispidus*, is the only species that has been reared to postlarvae in the laboratory (Fletcher et al. 1995). It is the most difficult species to culture in artificial conditions among all of the species of cleaner shrimps researched. It has a long larval duration (>120 days) (Fletcher et al. 1995). Generally, mortality during larviculture is very high, and it is difficult to grow larvae to postlarvae (Young 1979, De Castro and Jory 1983). Therefore, mass culture of the shrimp larvae has not been realized. Golden coral shrimp is also one of the most beautiful ornamental shrimps. To our knowledge, there is no report on the larval culture of the shrimp. In this study, we compared the effects of three common diets used in decapod larval rearing—*Artemia* nauplii, rotifer, microalgae *Chaetoceros gracilis* (Cg)—on survivorship and development of *S. scutellatus* larvae and undertook the experimental larviculture.

MATERIALS AND METHODS

The study was conducted at the Harbor Branch Oceanographic Institution, Inc., Fort Pierce, FL in 1996.

Broodstock

One pair of the shrimps (one male and one ovigerous female) was purchased from a local pet shop (collected from the wild 1 to 2 days before our purchase) and was maintained in a recirculating seawater system (in a 25-L plastic tank) under 14-h light:10-h dark in a greenhouse. The total length (TL) of the ovigerous female was 3.6 cm. Temperature fluctuated 4–6°C daily (between 26 and 32°C during the study period). Salinity was 33–35 ppt. The shrimps were fed in excess with frozen *Artemia* or squid once a day. The female that was going to hatch was moved to a 270-L conical fiberglass tank equipped with an internal standpipe with 53- μ m-pore-size mesh. After the female had hatched and molted, it was taken back to the 25-L tank. All larvae used in this study were from the same female.

Effect of Different Foods on Survival and Development

A batch of larvae was placed in 4-L bottles with 2.5 L of seawater (28–30 ppt salinity and 26.5–29°C temperature), with gentle aeration. Each bottle contained 20 zoea I larvae. Water exchange (50%) was conducted, fresh algae were used, and all *Artemia* nauplii and rotifer were renewed everyday.

There were three food treatments (each with three replicates), *Artemia* nauplii, rotifer, and Cg. Food density for *Artemia* nauplii was 5–10/mL, that for rotifer was 10–15/mL, and that for Cg was 50,000–100,000 cells/mL. All experimental bottles were arranged randomly. Survivorship and development were examined daily.

Larviculture

On the basis of the results of the food experiment (see the Results), we used *Artemia* nauplii as feed in our larviculture study.

The larviculture was conducted in 25-L plastic tanks, each with 200 larvae. The three trials were carried out with a temperature of 26–29°C and a salinity of 28–30 ppt. Larvae were fed with *Artemia* nauplii (5 nauplii/mL) with daily renewal. Water exchange rate was 50% daily. Survival was measured once every 5 days.

Data Analysis

One-way analysis of variance (ANOVA) was used to analyze survivorship of larvae fed with different diets, and the *T*-method multiple comparisons test was used to compare the means when ANOVA showed a significant effect (Sokal and Rohlf 1995). Homogeneous of variance was tested using Bartlett's test before ANOVA.

RESULTS

Effect of Different Food on Survival and Development

Larvae fed with *Artemia*, rotifer, and Cg died on Days 21, 12, and 11, respectively. On Day 12, survivorship of *S. scutellatus* larvae fed with *Artemia* nauplii was $78.8 \pm 2.5\%$ (mean \pm SD) (Fig. 1). The difference of survivorship was significant ($p < 0.01$, one-way ANOVA) after Day 6. On Day 6, survivorship of larvae fed with *Artemia* ($96.3 \pm 2.5\%$) was not significantly ($p > 0.05$, *T*-method) different from that fed with Cg ($95.0 \pm 0\%$), but survivorship in both treatments was significantly ($p < 0.05$, *T*-method) higher than that in the rotifer treatment ($80.0 \pm 4.1\%$). On Day 9, larval survivorship in *Artemia* treatment ($90.0 \pm 0\%$) was significantly higher ($p < 0.05$, *T*-method) than that in both rotifer ($71.3 \pm 4.8\%$) and Cg ($42.5 \pm 2.9\%$) treatments, and the survivorship in rotifer treatment was significantly higher ($p < 0.05$, *T*-method) than that in the Cg treatments. The larvae in *Artemia* nauplii and rotifer treatments molted in the same interval: 3.5 days from zoea I to zoea II and 4 days from zoea II to zoea III. Larvae fed with Cg developed slower and could not develop beyond zoea II stage.

Larviculture

The larvae in the first trial hatched from the eggs spawned before the female was collected from the natural environment. All of the other larvae for this study were spawned in captivity. Fifty-five postlarvae (27.5% survivorship) were obtained in the first trial. The first postlarva was obtained on Day 43, when the survi-

vorship of the larvae was 50% (Fig. 2). Larva survivorship on Day 43 in Trials 2 and 3 was 31.5 and 34.1%, respectively. In the first trial, all larvae and postlarvae died because of infection by *Zoothamnium* sp. In each of the other trials, only one larva developed into postlarval stage, on about Day 70.

DISCUSSION

Effect of Different Foods on Survival and Development

Algae are suitable food for penaeid shrimp larvae (e.g., Gopalakrishnan 1976, Tobias-Quinitio and Villegas 1982, Wilkenfeld et al. 1984), but not for *S. scutellatus* larvae. *Artemia* nauplii and rotifers are known to be suitable food for a variety of decapod larvae (McConaughy 1985). This study showed that newly hatched *S. scutellatus* larvae can consume *Artemia* nauplii. This is different from penaeid shrimp larvae, which can only consume *Artemia* nauplii when they reach zoea III, even mysis I stage. This may be because zoea I larvae of *S. scutellatus* (3.8 mm TL) are equivalent in size to mysis I stage of penaeid shrimps. Larvae fed with rotifers and microalgae have lower survival and/or a slower development rate than those fed with *Artemia* nauplii, probably because of the smaller size and/or less suitable nutrition content of rotifers and microalgae.

Food size is an important consideration in larval rearing (Frost 1972), as shown in seven decapod species where small algae only occasionally support larval development compared with larger algae (Harms and Seeger 1989). In decapod larvae, ingestion rate is normally low for small-sized food (see Grahame 1983 for a review). Insufficient food intake may be the main reason for the lower development or survival rate. We found that *S. scutellatus* larvae can grasp *Artemia* nauplii with their mouthparts. This may be more efficient for food intake than filtration, another important feeding method for small-sized foods in decapod larvae (see Grahame 1983 for a review). Larvae may also be able to select food on the basis of size. Larger and later development stage animals may take larger-sized food (see Grahame 1983 for a review). *Penaeus kerathurus* postlarvae did not ingest rotifers when *Artemia* were present (Yufera et al. 1984). Nutrition plays an important role in affecting larval development and survival (Levine and Sulkin 1984, Staton and Sulkin 1991). Food nutritional value is found to affect assimilation efficiency in crustaceans. Some crustaceans

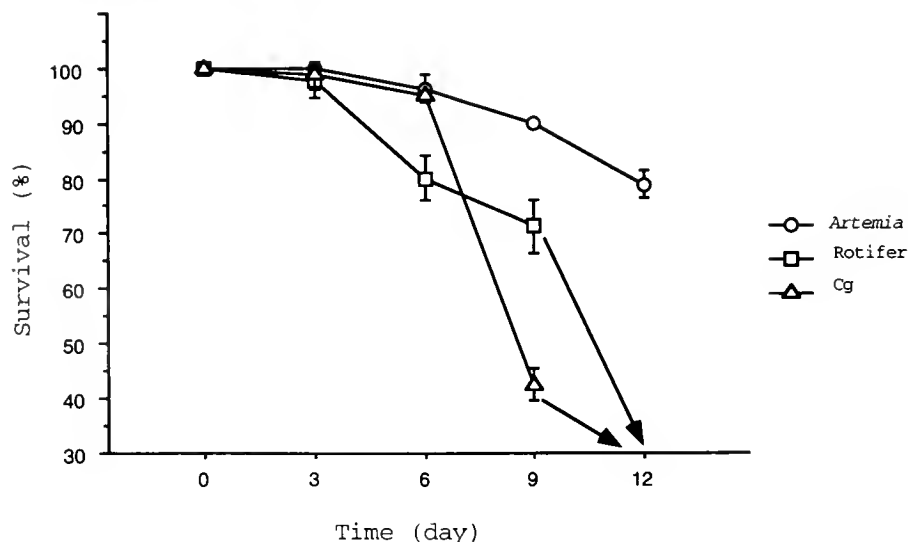


Figure 1. Survival of *S. scutellatus* larvae fed with different foods.

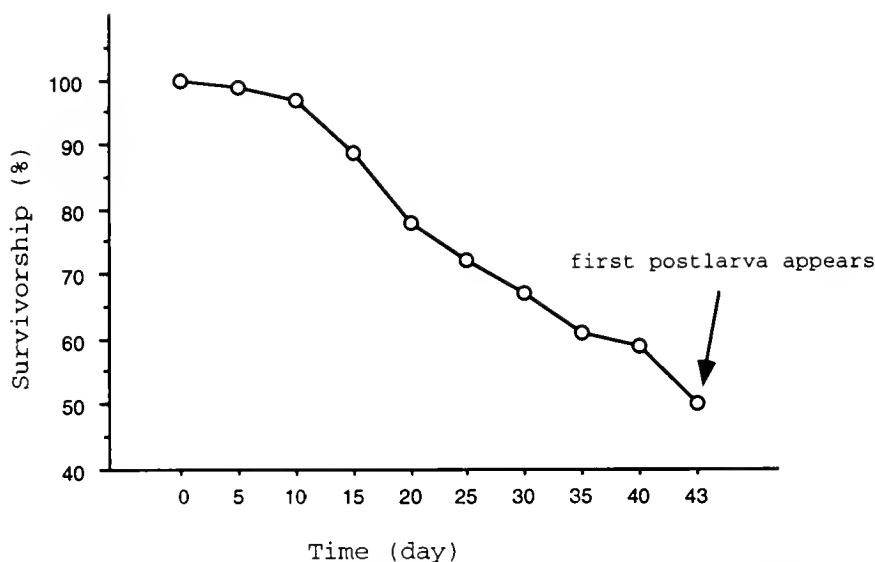


Figure 2. Survivorship of the golden and post larvae coral shrimp, *S. scutellatus*, larvae in the first trial. Fifty-five postlarvae were obtained. All larvae died because of infection by *Zoothamnium* sp.

show higher assimilation efficiencies on animal than on vegetation food (see Grahame 1983 for a review). Most decapod larvae have been considered to be exclusively carnivores.

Larviculture

Not only survivorship, but also metamorphosis rate from zoea to postlarva, was higher in the first trial, when the eggs were spawned in the natural environment, than in the subsequent two trials. Egg quality may be different between those spawned in nature and those spawned in captivity. Egg quality difference has

been found within a decapod species in different seasons in the wild (Amsler and George 1984), even within a brood (Clarke 1993). Improving rearing conditions, including broodstock nutrition, is expected to make mass culture possible for this popular aquarium species.

ACKNOWLEDGMENTS

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THE CHILEAN ARTISANAL STONE CRAB (*HOMALASPIS PLANA*) FISHERY: CATCH TRENDS IN OPEN ACCESS ZONES AND THE EFFECT OF MANAGEMENT AREAS IN CENTRAL CHILE

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ABSTRACT The Stone crab *Homalaspis plana* supports an important artisanal fishery along the coast of Chile. The objectives of this study were to analyze the trends in crab catches and CPUE between 1991 and 1994 in open access fishing areas of Central Chile and to compare the size and sex composition of the catches for two alternative fishing gears. We explored three different CPUEs, because abundance indicators have not been used before for this fishery. In addition, we compared the CPUE, the crab size distribution, and the sex ratio between open access fishing grounds and Management and Exploitation Areas (private grounds). Stone crab catches decreased between 1991 and 1994 in open access areas. The Stone crab is caught with crab pots and by divers, and no differences in mean crab size were found between fishing gears in El Quisco. The proportion of males caught in crab pots is higher than that caught by divers, and the proportion of ovigerous females was lower in crab pots. The CPUE (catch per trip) also decreased between 1991 and 1994 in open access fishing grounds. We analyzed alternative CPUEs that could be used for crab as well as for other benthic species. We show that the CPUEHours (catch per hour) is affected by the number of species caught, which suggests the importance of taking this factor into account. The CPUETarget (catch per hour corrected by the number of target species) is not affected by the number of species caught (target and/or bycatch) because this estimator considers the time allocation for the main species collected. The latter may be a more appropriate indicator. No differences in CPUE between open access grounds and Management and Exploitation Areas (private grounds) were observed. The size distribution of crabs in open access fishing grounds and in Management and Exploitation Areas was not significantly different; females predominated in both areas (>90%). Previous studies conducted in Management and Exploitation Areas focused on sessile or sedentary species and clearly showed the effect of human activity (removal) on the abundance and size of exploited species, compared with open access zones. The lack of differences in CPUE, crab size, and proportion of sexes between open access zones and Management and Exploitation Areas suggests that mobile species may offer a new challenge to the management tools recently implemented by the Chilean Fisheries Administration.

KEY WORDS: *Homalaspis plana*, crab, fishery, artisanal, Chile

INTRODUCTION

The Stone crab *Homalaspis plana* (Milne-Edwards, 1934) is distributed from Guayaquil (Ecuador) to the Magallanes Strait and Isla Juan Fernández, Chile (Garth 1957), between the intertidal zone and 272 m (Henriquez and Bahamonde 1976). This crab species supports an important artisanal fishery along the coast of Chile. The fishery is open all year without any limitation in total catches; the only regulations are size limit (>120 mm carapace width) and ovigerous females (Bustamante and Castilla 1987). The current status of the Chilean Stone crab population is unknown, because of the lack of systematic catch statistics. Furthermore, the proportions of ovigerous females and undersize crabs caught are also unknown, because regulations are not properly enforced.

The major gaps in understanding the multispecies artisanal fisheries in Chile are the extreme interest for locos (*Concholepas concholepas*) and the lack of population abundance indicators. The simultaneous catch of several invertebrate species in each fishing trip presents difficulties for the estimate and use of abundance indicators. Besides, information on catch and effort is rarely available for most of the species targeted by the artisanal fishery. One of the few exceptions is Caleta El Quisco (33°23'S, 71°42'W), located in Central Chile, where catch and effort data for the main species exploited started to be collected in 1991, as a new Management and Exploitation Area (MEA) was created. Most of the catch statistics come from open access fishing grounds, because the MEA is closed to the fishery. Since 1991, the MEA was

opened only to the loco fishery during the ban lifting, and crabs were first and only harvested in 1995 during a limited time (1 day).

This work is the first approach to analyze the artisanal, Stone crab fishery in Chile. The study was conducted in open access and closed fishing areas in Central Chile, and the objectives were as follows:

- (1) to examine the trend in catches and CPUE at the local scale (Caleta El Quisco) between 1991 and 1994. We conducted an exploratory analysis in order to obtain an indicator of crab abundance for the multispecies, artisanal fishery. We think that the CPUE indicators explored here are also of importance for their potential use for other benthic resources. We also think that the analysis of catch data at a local scale may have implications on a larger scale, because the same fishing gear is used along the coast of Chile.
- (2) to compare the catch composition (crab size and sex) between two fishing gears (crab pots and divers). This analysis is of interest to assess the effect of each fishing gear on sublegal—size individuals and ovigerous females.
- (3) to study the effect of the MEA on the size structure and sex ratio of the Stone crab population. This result is relevant from the perspective of the new management tools recently implemented in Chile. The use of the MEA is a novel, alternative management strategy still under experimentation (Payne and Castilla 1994, Castilla et al. in press). The implementation of the MEAs was based on studies conducted in a marine reserve in Central Chile on sessile and

sedentary species (Castilla and Durán 1985, Durán et al. 1987, Durán and Castilla 1989, Castilla and Bustamante 1989, Castilla 1990, Oliva and Castilla 1990, Oliva and Castilla 1992). However, some of the benthic species targeted by the MEA are highly mobile, among them our study species, the Stone crab.

MATERIALS AND METHODS

Study Site

Our study site is located in Caleta El Quisco, Central Chile (Fig. 1). In Chile, fishermen are organized in unions within Calatas. The El Quisco Union is very well organized and took legal possession of an MEA in 1993 (for details about MEAs, see Payne and Castilla 1994, Castilla and Pino 1996, Castilla et al. in press). However, the union had totally banned (unilaterally) diving activities on a coastal area of 57 ha of sea bottom (3 ha intertidal, 54 ha subtidal) 2 years before (1991). Since then, this area has not been exploited for most benthic resources, except for three extractions of locos during the ban lifting for this species (Payne and Castilla 1994, Castilla et al. in press).

Open access fishing grounds exploited by fishermen of El Quisco are located up to 60 min sailing time north or south from the landing harbor and the El Quisco MEA (EQ; Fig. 1, Table 1). The southern fishing grounds are located near another MEA, assigned to the Fishermen Union of Las Cruces in 1993 (LC, Fig. 1).

The MEA of Algarrobo is located toward the north. (A, Fig. 1). Fishermen of Las Cruces do not comply with the fishing restrictions for the MEA as in El Quisco and Algarrobo.

Data Set

Catch data for all of the species landed in El Quisco have been recorded since 1991 by the Fishermen Union. Catch data are reported by species (in numbers and/or weight) per trip; for the Stone crab, catches are reported in numbers. We investigated the fishing trips in which crab was the only or among the most important species caught. The information about other species caught is not presented here but was used to estimate CPUE (see section c). Effort data consisted of total time spent at sea (diving time was not available in the data set). The fishing grounds visited were also recorded. Crab pots were not included in this analysis because this fishing gear has recently been introduced.

CPUE Estimates

One of the main problems faced by the managers of the artisanal fishery in Chile is the difficulty in estimating abundance indicators. On the basis of the data available in El Quisco, we explored several crab abundance indicators that could also be applied to other species targeted by the artisanal fishery. This analysis was conducted to assess the value of different indicators of crab abundance.

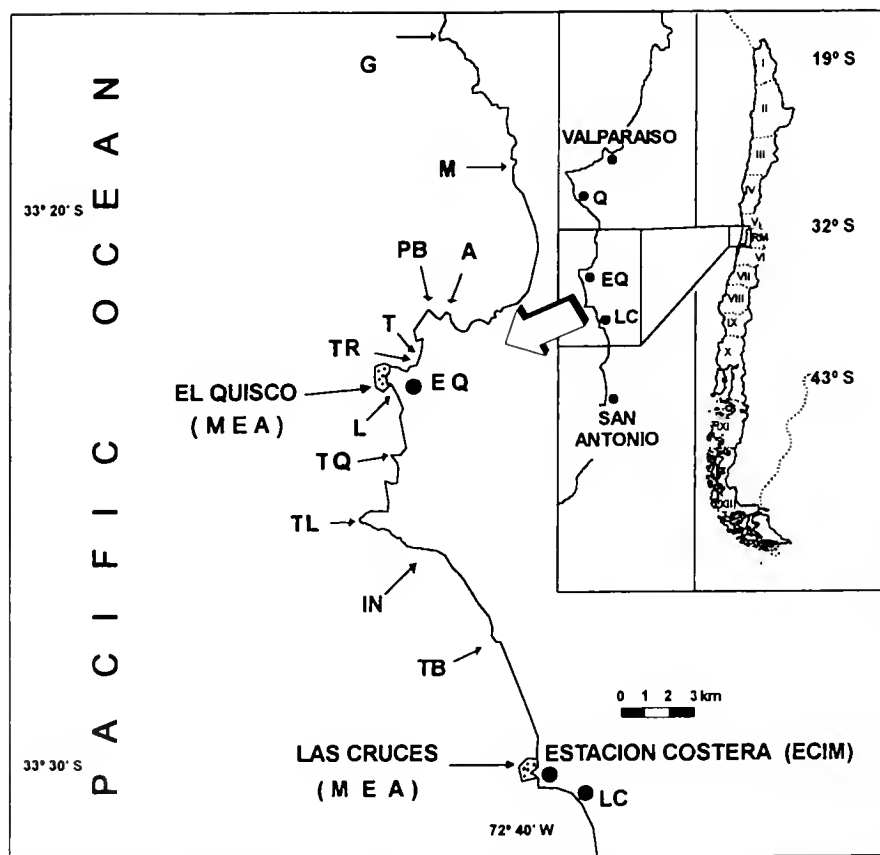


Figure 1. Map showing the regions for which catches are reported and fishing grounds for the study area in Central Chile. The codes used for fishing grounds indicate: G (El Gallo), M (Mirasol), A (Algarrobo), PB (Peña Blanca), T (Los Toros), TR (Toribio), EQ (El Quisco, landing location), L (Los Lobos), TQ (Tablaque), TL (Punta Tralca), IN (Isla Negra), and TB (El Tabo). Shaded zones indicate MEAs (El Quisco and Las Cruces). Dots indicate coastal towns and the Marine Reserve of Las Cruces. Q, Caleta Quintay MEA.

TABLE 1.

Most common fishing grounds used by fishermen of El Quisco (explored more than six times) between 1991 and 1994.

Fishing Grounds			1991		1992		1993		1994	
Location	S. Time	Weather	% Trips	CPUE	% Trips	CPUE	% Trips	CPUE	% Trips	CPUE
Gallo	60 N	7	0.8		10.1	192.0	0.7		5.6	235.0
Mirasol	50 N	20					8.7	91.7	12.7	99.7
Peña Blanca	15 N	10	4.3	102.5	4.2					
Los Toros	10 N	20	5.4	94.4	7.4	127.5	0.7		1.4	
Toribio	10 N	19	8.2	98.0	10.2	115.5	5.3	108.7	18.3	56.0
El Quisco	0	no data	7.8	89.7	9.1	76.8	10.0	86.0	8.4	68.0
Lobos	20 S	21	10.1	82.7	11.6	102.8	10.7	113.0	8.4	50.1
Tablaque	30 S	20	3.1	101.5	4.9	116.1	8.7	121.5	11.3	74.4
Punta Tralca	40 S	19	16.7	121.5	12.9	153.6	16.7	111.7	23.9	93.2
Isla Negra	50 S	13	11.3	107.3	10.9	114.9	24.0	108.3	7.0	62.4
El Tabo		13	31.5	120.9	17.9	123.6	14.7	94.5	2.8	100.0

Sailing time (minutes) to fishing grounds located north or south from the harbor (El Quisco) and average number of days with favorable weather conditions for fishing operations are reported. Both were estimated on the basis of a survey conducted among fishermen. The percentage of trips made to each ground per year and CPUE are also reported.

The simplest abundance indicator used was catch per trip (hereafter, CPUE). The second abundance indicator uses hours at sea as effort (CPUEHours). The number of species caught per trip varied; divers collect not only crabs, but also other species (e.g., limpets, loco, sea urchins). The species diversity of the catches may be due to random opportunities during diving and/or sales opportunities (specific targets). Thus, several species may be caught per hour or trip, but only some of them can be considered target species. The remaining species can be considered bycatch (they represent a minimum proportion of the catch). Taking into consideration the way the fishery operates, a third indicator of abundance was estimated (CPUETarget) using the number of target species and hours at sea (crabs/[hours/number of target species]). This estimator assumes equal time allocation for each species targeted.

In order to determine what could be considered a target species, we classified the number of species caught into four categories: (1) other species was/were the target, and crabs were the bycatch; (2) crabs were the target, and all other species collected were the bycatch; and (3) two species were targeted (crabs plus another). We determined if a species was a target or bycatch based on our knowledge of "minimum" average potential catches by species, when that species is the target. Species were considered bycatch if the diver had collected: <15 kg of fish, <50 kg of tunicates (*Pyura chilensis*), <30 kg of limpets (*Fissurella* spp.), <100 sea urchins (*Loxechinus albus*), <30 kg of mussels (*Mytilus chilensis* and *Choromytilus chorus*). Species were considered target if (1) they dominated the catch (catch twice as high as the limit used to consider that species bycatch), and/or (2) for crabs, more than 50 crabs per trip were caught.

Two-way analyses of variance (ANOVAs) were used to test the effect of number of species caught (up to three) and time (years) on the different types of abundance indicators (CPUE, CPUEHours, and CPUETarget). Natural log transformations were used in CPUEHours, to correct for heterocedasticity. Multiple range tests (LSD approximation) were used for a posteriori comparison across means. The trend in catches and CPUE in Caleta El Quisco were examined on a monthly and annual basis. We identified the fishing grounds where the fishery operated between 1991 and 1994, analyzed trends in the exploitation of different fishing grounds, and

discussed factors that could produce a differential use of effort in space.

The Effect of Fishing Gears

The Stone crab is mostly caught using two fishing gears: crab pots and divers, operating either from fishing boats or from the shore. Shellfish food gatherers that operate in the intertidal zone may also collect crabs (mostly unreported catches), but we have focused our study only on those fishing gears that account for most of the catches. Crab pots were introduced in Caletas El Quisco and Las Cruces as an alternative fishing gear in 1994.

Crabs landed in Caleta El Quisco (EQ, Fig. 1) and Caleta Las Cruces (LC, Fig. 1) between October 1994 and February 1995 were measured and sexed, in order to compare the catch composition (crab size and sex ratio) between two fishing gears (crab pots and divers). We report the proportion of ovigerous females, sexes, and undersized crabs.

Crab Size and Sex Ratio in Open Access Zones and MEAs

In April 1995, the El Quisco MEA (Fig. 1) was opened for a 1-day extraction. This event allowed us to study the effect of the Management and Exploitation Areas for Benthic Resources on the Stone crab population size structure and sex ratio.

During the ban lifting, there were 42 registered divers in El Quisco Union. Each fishermen had a quota for three species; the quota for the Stone crab was 50 crabs per diver. Eighteen divers extracted the quota (or part); catch per diver, crab size, and sex were recorded. We estimated CPUE in the El Quisco MEA. Several resources were extracted simultaneously, and when there were multiple targets, crabs were usually the bycatch. Thus, the comparison of CPUE (catch per trip) considering the effect of multi-species targets was not possible. However, in some cases, only crabs were harvested, and the CPUEdive (catch per unit of diving time) was estimated. No statistical comparisons between CPUEs in open access fishing grounds and MEA were conducted, because of the differences in the abundance indicators. The CPUEHours is expected to produce lower estimates, because sailing and han-

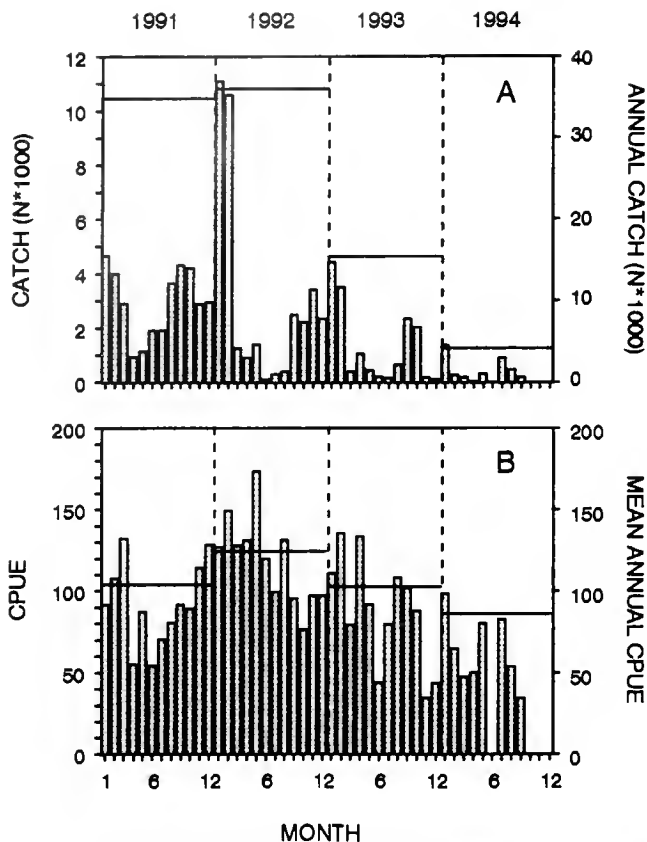


Figure 2. (A) Monthly crab catches (numbers) between 1991 and 1994 landed in El Quisco; horizontal lines (right y-axis) indicate total annual catch. (B) Monthly CPUE (catch in numbers per trip) between 1991 and 1994; horizontal lines (right y-axis) indicate mean annual CPUE. The CPUE was calculated by pooling the catches for different numbers of target species.

ding time were included, whereas only diving time was used for CPUE_{Dive}.

Crab size distribution, mean size by sex, and sex composition were compared between open access zones and the MEA of El Quisco. Only one fishing gear was compared (divers). Crabs were also measured in open access zones and in Las Cruces MEA (Fig. 1) between October 1994 and April 1995. In order to avoid confounding effects due to fishing gears, the most frequent fishing gear in Las Cruces (crab pots) was chosen for the comparison. Although depth may account for differences in sex ratio, it was not included in this analysis.

RESULTS

Exploratory Analysis of Alternative Abundance Indicators and Trends in Catches and CPUE Between 1991 and 1994

There was a sharp decline in total crab catches in Caleta El Quisco between 1991 and 1994. Annual catches were similar in 1991 and 1992 (35,416 and 36,526 crabs, respectively), but the monthly pattern differed (Fig. 2A). In 1991, the monthly distribution of the catches was more homogeneous than in 1992, when most of the activity occurred in January and February (Fig. 2A). In 1993, the annual catch decreased more than 50%, and a 10-fold decrease occurred between 1991 to 1992 and 1994. The price did not vary between 1991 and 1994. Although a decrease in the

catches may have occurred, there was also a large proportion of unreported catches in 1994.

The annual CPUE also showed a declining trend, although the decrease between 1992 ($\bar{X} = 129.4$) and 1994 ($\bar{X} = 82.6$) was 36% (ANOVA: $F = 7.3$, $df = 3,668$, $p < 0.0001$; Fig. 2B). The number of species caught did not have any effect on CPUE (ANOVA: $F = 2.5$, $df = 2,668$, $p = 0.08$; Fig. 3A). The CPUE_{Hours} was also significantly different across years (ANOVA: $F = 9.8$, $df = 3,668$, $p < 0.00001$) and was affected by the number of species caught (ANOVA: $F = 13.3$, $df = 2,668$, $p < 0.00001$; Fig. 3B). The lowest CPUE_{Hours} corresponded to 1994 and 1991, and the highest corresponded to 1992 and 1993 (Fig. 3B). The CPUE_{Hours} was lowest when three species were caught, intermediate when two species were harvested, and highest when only crabs were caught. The CPUE_{Target} was also significantly different across years (ANOVA: $F = 7.6$, $df = 3,668$, $p = 0.0009$; Fig. 3C). The highest estimates were found for 1992 and 1993, and the lowest were found for 1991 and 1994 ($p < 0.05$). The CPUE_{Target} was not affected by the number of species caught ($F = 2.35$, $df = 2,668$, $p = 0.1$).

The CPUE_{Hours} was also compared across months for the 4 years. Although there were statistically significant differences within year, no clear, consistent pattern across years was found (Fig. 2B). In 1991, several homogeneous groups were detected; the highest CPUE_{Hours} were found between August and March, and

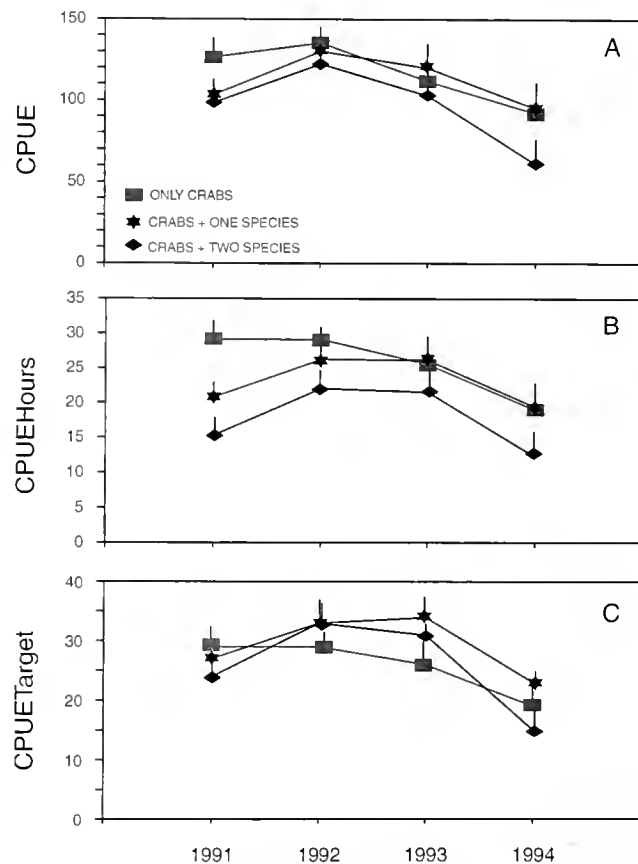


Figure 3. Abundance indicators estimated for El Quisco area combining all fishing grounds and divers between 1991 and 1994 when only crabs are the target species and when two and three species are targeted (including crabs). (A) CPUE (catch per trip); (B) CPUE_{Hours} (catch per hour at sea); (C) CPUE_{Target} (catch per hour at sea corrected by the number of target species).

the lowest were found in the winter (June and July). Surveys conducted among fishermen of El Quisco showed that fishermen observed the lowest abundance of crabs in the winter. In 1992, the highest CPUEs were found between January and May, and the lowest were found between September and December. No clear patterns were found in 1993 and 1994.

The pattern of CPUE among fishing grounds was also explored. The rationale for this analysis was that fishing activities in the vicinity of the MEA may affect the abundance of mobile species in the closed areas. In 1991 and 1992, there was a tendency to exploit some fishing grounds located toward the south of the landing site (mostly Punta Tralca and El Tabo; Fig. 1 and Table 1), whereas in 1993 and 1994, the preference was not clear and fishing grounds located toward the north, or closer to the landing site (EQ), were also fished (Table 1). The fishing ground of El Gallo and Peña Blanca showed a low percentage of fishing trips, probably because of the low probabilities of favorable weather conditions (Table 1). The patterns of interest that appeared from this analysis were that: (1) the most productive fishing grounds (TQ, TL, TB; Table 1) are located toward the south, where the highest percentage of trips were reported; (2) multispecific targets were found in the most productive fishing grounds of Punta Tralca, Tablaque, and El Tabo; and (3) the three fishing grounds located closer to the marine reserve are mostly monospecifics, crabs being the main species harvested. A decrease in CPUE was observed in these fishing grounds in 1994 (Table 1). The three abundance indicators were also compared across years between the two most visited, and also very productive, fishing grounds: Punta Tralca and El Tabo. No differences were found in any of the comparisons (p always >0.05).

The Effect of Fishing Gears

Mean size, the proportion of undersized individuals in the catch, the sex ratio, and the proportion of ovigerous females were estimated for catches landed in Las Cruces and El Quisco between October 1994 and February 1995. In El Quisco, mean crab size ranged between 111.7 (standard deviation [SD] = 10.3, crab pots) and 112.9 (SD = 10.7, diver, ANOVA: $F = 0.4$, $df = 2,736$, $p = 0.69$), and only 25% of the catch was composed of legal-sized individuals (>110). The percentage of males caught in crab pots was higher (13%) than that caught by divers (4–6%), whereas the proportion of ovigerous females was lowest in crab pots (0.06%), intermediate for hooka-divers (8%), and highest for coastal divers (43%).

The main fishing gear identified in Las Cruces was crab pots; they can be deployed from a boat or by skin divers from the shore. Mean crab size was smaller when the crab pots were deployed by divers from the shore ($\bar{X} = 94.9$, SD = 9.7; ANOVA: $F = 158.6$, $df = 1,390$, $p < 0.00001$). Most of the catch was composed of undersized individuals (99%) and a high proportion of males (26%); no ovigerous females were caught. Mean crab size was larger for crab pots operated from a boat in the same fishing ground (108.2, SD = 10.9); 14% of the catch was composed of legal-sized individuals. The proportions of males (24%) and ovigerous females caught (0.09%) were similar to those found in the shallow subtidal crab pots.

Comparison in Catches and Size Between Open Access Zones and the MEAs

The size distributions of crabs collected by divers in open access zones and the MEA of El Quisco were not significantly dif-

ferent (KS: DN = 0.13, $p = 0.198$; Fig. 4A and B); females predominated in the MEA (90.6%) and open access zones (94%). The same pattern was observed in Las Cruces (KS: DN = 0.15, $p = 0.18$; Fig. 4C and D), although more males were present (between 17% in the MEA and 23% in open access zones). The size frequency distribution was also significantly different between El Quisco ($\bar{X} = 111.7$, SD = 10.29) and Las Cruces ($\bar{X} = 108.1$, SD = 10.9, KS: DN = 0.22, $p < 0.0001$). Crabs harvested with crab pots in both sites were used for the latter comparison.

During the one-time harvest of crabs, conducted in April 1995 in El Quisco, it was possible to estimate catch per hour of diving (CPUEDive). The average CPUEDive was 36.03 (crabs per hour when crabs were the only target, SD = 26.05) and varied between the subzones of the MEA. When two species were targeted, the CPUEDive was 42.5 (SD = 24.4), but the number of species targeted had no effect on the CPUEDive (ANOVA: $F = 0.27$, $df = 1,24$, $p = 0.61$).

DISCUSSION

The Stone crab represents an important resource for the Chilean artisanal fishery, comparable with the current landings (in tons) of limpets, locos, and some species of clams (SERNAP). On the basis of the data available at the national level, the Stone crab is the third most important crab species exploited in Chile, accounting for 13–16% of crab annual catches (SERNAP). The Stone crab is the

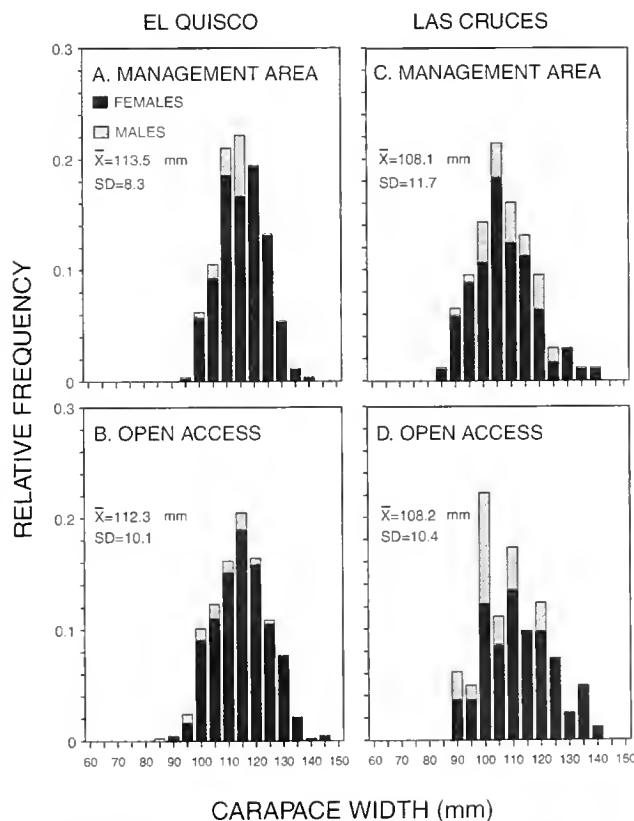


Figure 4. Size distribution by sex in two MEAs: (A) El Quisco and (C) Las Cruces and nearby open access fishing grounds; (B) El Quisco and (D) Las Cruces. In Las Cruces, only crab pots were used; in El Quisco, only crabs collected by divers were considered.

most important crab species harvested between the III and IV Regions and among the most important crab species in our study area (V Region). Annual catches of El Quisco represented 10% of the catches in the V Region in 1991 and 15% in 1992 and decreased to 7 and 3% in 1993 and 1994, respectively.

Between 1991 and 1994, there was a decrease in the percentage of Stone crab versus other crab species caught in the V region (SERNAP) and in El Quisco. On the basis of samples conducted in just one location in Central Chile (El Quisco), we could observe that the fishery statistics may show a dramatic underestimation of the current catch levels of the Stone crabs, as well as of other species. We compared the crab data that we collected between October 1994 and April 1995 with those recorded by the Fishermen Union, in order to estimate if the observed decreasing trend in catches was related to unreported cases. The proportion of unreported catches cannot be compared over time, but between October 1994 and April 1995, 85% of the crab catches were not reported and similar observations were made for other species. El Quisco is probably one of the locations where catch statistics are more reliable. A high proportion of unreported catches may not be exclusive to the Chilean artisanal fishery, but we think that it is important to emphasize that the actual crab catches may be much higher than official reports.

The main characteristic of the Chilean artisanal fishery is the diversification of fishing effort. Hooka-divers target on several benthic invertebrates, depending on fishing and sales opportunities. Despite the low capacity to improve the fishing efficiency in this artisanal fishery, and the diversification of effort among species, some species have been overexploited (e.g., loco, Castilla et al. in press), others showed dramatic reduction in size (e.g., keyhole limpets, Pino and Castilla 1995; sea urchin, Castilla and Pino 1996), and the Stone crab in Central Chile shows a decline in CPUE. Stock assessments have been conducted for some resources at the national level (e.g., loco, sea urchin), and quotas or bans applied in some cases. However, the current level of exploitation of several benthic resources in Chile is unknown and poorly regulated. In fact, in our study area, most of the Stone crab landings are composed of illegal-sized individuals (more than 75%). Similar percentages have been reported for other locations in Central Chile (Mendoza et al. 1994). On the basis of the limited data available about the biology of the species, females could reproduce only once before reaching the legal size. Considering the low proportion of males in the population, and the high proportion of undersize individuals caught, it can be suggested that some females may enter the fishery even before reproducing.

In all of the locations sampled, the proportion of males is remarkably low and is comparable to observations made in southern Chile. In other MEAs of the V Region, however, a high proportion of males has been found (1:1 to 2 male:1 female, Mendoza et al. 1994). Differences in sex ratios between El Quisco and Las Cruces may be due to the different fishing gears used among locations but, in any case, reflect a low proportion of males. However, on the basis of the information available, it cannot be stated that the differences in sex ratio are due to harvesting. The Stone crab uses shelters in rocky habitats, which may be a limiting resource. A potential for polygynic mating systems exists when males monopolize a limiting resource (Emlen and Oring 1977). Thus, the high proportion of females may also be explained by the mating system of this species. Carvacho et al. (1995) stated that a polygynic mating system in the Stone crab can be suggested by the early chelae development in males.

The estimation of abundance indicators for the Chilean artisanal fisheries has been challenged by the difficulties in obtaining reliable catch statistics, by the lack of effort data in most cases, and also by the multiple invertebrate species targeted (Bustamante and Castilla 1987). Here, we present alternative CPUEs that could be used for crab as well as for other benthic species. We show that the CPUEHours is affected by the number of species caught, which suggests the importance of taking this factor into account. The CPUETarget is not affected by the number of species caught (target and/or bycatch) because this estimator considers the time allocation for the main species collected. The latter may be a more appropriate indicator, because otherwise, the abundance indicator may underestimate the actual abundance, or show higher variability depending on the differences in number of species caught. It is worth noticing that the CPUETarget is higher when two or more species are collected. It may be because the most productive fishing grounds (highest CPUE) are multispecific, and time is optimized by harvesting several species than by searching for one specific target.

Although fishermen observation and CPUE trends indicate that, overall, the crab stock is declining, this tendency could not be observed at the fishing ground scale. The effect of harvesting on the most visited fishing ground could not be statistically detected on the basis of CPUE or CPUEHours until 1993. However, there was a trend to exploit more crabs near the MEA in 1993 and 1994, and in those specific fishing grounds, the CPUE decreased in 1994. This pattern of exploitation may have an effect on the crab abundance in the MEA, which does not show an increase in abundance as in sessile species. The CPUEDive estimated when crabs were the only target was comparable to that estimated in open fishing grounds (36.03 in the MEA, and from 17 to 29 in open fishing grounds). It should be noticed that the CPUE for the MEA was estimated using diving time as effort rather than total time (which includes diving, handling, and sailing times).

Size and sex ratio data collected in open fishing grounds are comparable to those recorded for the MEAs of El Quisco and Las Cruces. It is worth noticing, however, that the harvest strategy and the fishing gear in both locations are completely different. El Quisco MEA has been closed for the last 3 y, whereas Las Cruces MEA could be considered as an open access fishing ground. Thus, the effect of harvesting on crab size could not be observed, irrespective of the management strategies for the MEAs.

The lack of differences in crab size, sex ratio, and CPUE between open access fishing grounds and MEAs, and the lack of harvest effect at the fishing ground scale, suggest that the mobile characteristic of crabs may set new challenges to the new fishing strategy implemented by the Chilean Fisheries Administration. The use of MEAs was based on studies about the human effect on the intertidal community. Those studies, mostly directed toward sessile or relatively sedentary species, clearly showed the effect of human harvesting on species abundance and size (Castilla and Durán 1985, Durán and Castilla 1989, Durán et al. 1987, Castilla 1990, Oliva and Castilla 1990, Oliva and Castilla 1992). Recent studies conducted at El Quisco showed higher CPUE and size of sea urchins, keyhole limpets, and locos located in the MEA compared with the open access fishing ground (Pino and Castilla 1995, Castilla and Pino 1996, Castilla et al. in press). However, this pattern was not observed for crabs.

The underlying assumptions of the new Fisheries Law are that the MEAs can maintain juvenile and adult production of several species and contain enough adults to export larvae to surrounding

areas. Higher production of adult crabs has not occurred in El Quisco MEA. Furthermore, studies currently underway to examine habitat requirements of juvenile Stone crab have shown that El Quisco MEA ranked among the lowest in juvenile habitat quality, because extreme exposure to wave impact (M. Fernández unpublished). Thus, two of the underlying assumptions of the MEAs have not been met in our study area for the Stone crab.

Our analysis shows that Stone crab abundance is declining in the vicinity of El Quisco and that the MEA of El Quisco may not meet the restocking objective for this species. Despite the ambiguous perspective of the use of MEAs as a management strategy of mobile benthic resources, this study represents the first approach to address this issue. We think that the new comanagement tools implemented by the Chilean Fishery and Aquaculture Law may provide an opportunity to study the Stone crab population, not only

contrasting the MEAs to open access zones, but also allowing for experimentation.

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BREEDING SUCCESS OF LARGE MALE RED KING CRAB *PARALITHODES CAMTSCHATICUS* WITH MULTIPAROUS MATES

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ABSTRACT Most multiparous red king crab *Paralithodes camtschaticus* (Tilesius, 1815) hatch their eggs during the brief spring diatom bloom; then, they molt and breed during a period of ≈ 20 days. This study examined the percentage of cleaving eggs in clutches of multiparous red king crab mated to males 140–204 mm carapace length (CL). Ten males had access to three or four females, and the intervals between individual matings ranged from 1 to 22 days. There was no obvious relationship between breeding success and the time interval between matings. Fertilization of the first females' clutch was successful for all 10 test males, with 97–100% of the eggs initiating division. Nine males bred their second potential mate; one did not. That female ovulated with another male, so she was fertile. Egg division rates ranged from 86 to 100% for the second matings. All 10 males fertilized the third female, with 5–100% of the eggs starting division. Only 66% of the males fertilized a fourth clutch, and egg division rates were 79–100%. One female fourth in line to be bred extruded a clutch in the presence of the test male, but none of the eggs divided. Two of the fourth mates had to have fresh males put in with them before they ovulated. The results suggest that most male red king crabs ≥ 140 mm CL can fertilize three mates during the brief period when most multiparous females breed.

KEY WORDS: king crab, reproduction, ovulation, fertilization

INTRODUCTION

Male red king crab *Paralithodes camtschaticus* previously supported an important commercial fishery in Alaska. Currently, several fishing areas have restricted harvest quotas because of low crab abundance. The reasons for the large-scale population decreases are unknown, but their occurrence has increased the desire to understand the biology of the species. The fishery is restricted to males 119–175 mm carapace length (CL) depending on location; in the area where the study specimens were captured, it was 178 mm when there was a fishery (Donaldson and Donaldson 1992). Because fishing decreases the number of large males, it is important to understand their reproductive capacity so stocks can be preserved. Males have been reported to mate 13 successive times, but their mating ability decreased after the sixth or seventh mating (Powell and Nickerson 1965, Powell et al. 1972, Powell et al. 1974). Knowledge of reproductive capacity is important to understand population dynamics. In one of the population models for red king crabs, it was assumed that large males can mate with up to three females during the peak of the breeding season (Zheng et al. 1995). The model assumed that most of the matings take place within a 20-day period because eggs typically hatch in synchrony during the brief spring phytoplankton bloom so that the first-feeding larvae may graze on diatoms (Paul et al. 1990). This experiment determined egg fertilization rates when three to four females were held with males 140–204 mm CL to test the model's assumptions that males could fertilize three mates in 20 days.

MATERIALS AND METHODS

Crabs for this study were captured near Homer and Kodiak, AK, by biologists from the Alaska Department of Fish and Game. There were 10 (6 Homer, 4 Kodiak) males and 39 (23 Homer, 16 Kodiak) females. They were captured with standard king crab pots at 50–60 m of water in March or April, just before the breeding season. Crabs were transported by truck from Homer, or by air from Kodiak, to the laboratory. The seawater for the Seward laboratory comes from below the pycnocline of a deep fjord, and its

temperature during the study was 3–6°C. Salinity ranged from 31 to 33 ppt. The tank size used in breeding experiments was 1000 L, and the water exchange rate in tanks was 100% per h. All test animals were held in separate tanks to prevent cannibalism. They were fed herring *Clupea pallasi* (Valenciennes, 1847) tissue and *Octopus dofleini* (Wulker, 1910) alternatively every other day.

Previous work demonstrated that for 10 days after molting, red king crab males are incapable of mating (Powell et al. 1974). Males used in breeding experiments were all hard old-shell status with worn spines. All females had eyed eggs, so they were multiparous; the first hatching occurred two weeks after capture. CL was measured for every crab used in breeding experiments. This measurement is taken from the right eye notch to the central portion of the rear margin of the carapace. Right chela height was measured at the point of maximum width. The 10 test males were 140–204 mm CL.

In observations of reproductive success, a male was put into its tank soon after capture and newly molted females were placed with him when they were available. We recorded the time intervals between matings, which varied because the timing of female molt was not controllable. All females were moved into the male tanks within 12 h of molting. Three or four multiparous females were available to each test male as potential mates. Female CL was recorded, and each was individually identified with a plastic numbered tag held on a leg with a cable tie. In all cases, the male CL was the same or larger than that of the female.

All copulations in this study occurred during April. Females usually ovulate within 24 h after molting, but each test male was held for 4 days with a newly molted female to see if a clutch would be produced in his presence. If ovulation was not observed within 4 days of a female's molt, another male, that had not bred any other females, was put into the tank. The original male, that did not breed with the test female, was removed before the new one was put into the tank. The new males were moved to the female's tank to minimize any "tank effect" on the female. Then, if a female produced a viable clutch with the second fresh male, the interac-

tion with the first male was considered a reproductive failure for him. Males were considered to be successful parents if the female extruded a viable clutch in his presence within 4 days of her molt.

After ovulation, females were isolated and held until their eggs developed to the 64- to 128-cell stage. Then, a group of eggs was randomly removed from each of the six pleopods and 100 of them were examined under a microscope for cell division. The site of collection of eggs on the pleopods was randomly selected each time an egg sample was taken. Values from the six subsamples from each female were averaged to estimate the percentage of dividing eggs in her clutch. Clutch size was qualitatively estimated as full, 3/4, or <3/4 full.

RESULTS

Table 1 provides the summary of the fertilization observations. All 10 females that were bred first in the test groups ovulated with 97–100% of the eggs cleaving. When the second group of females molted, nine of them bred with test males and they had 86–100% egg division rates. One of these females did not ovulate until a fresh male was moved into her tank, after which she produced a normal clutch with 99% of the eggs initiating division. The third group of females to molt all ovulated after copulating with their respective test males. Only 5% of the eggs in one clutch from a third mating initiated division, whereas in the other nine clutches, 98–100% of their eggs cleaved. There were only enough females to test nine males with a fourth breeding. Only six of those females produced viable clutches with test males as parents. In those successful fertilizations, 79–100% of the eggs had begun division. One female from the fourth group produced a clutch in the test male's tank; the eggs attached to pleopods, but none of them cleaved, indicating a fertilization failure. Two others from the fourth group had to have fresh males before they ovulated and produced a viable clutch. All females had egg clutches $\geq 3/4$ full.

The longest period in which any male had to breed all four females was 26 days for the first male, whereas the last one had to breed them all within 4 days (Table 1). The interval between individual matings ranged from 1 to 22 days. In the 10 instances when copulation occurred within 24 h of the previous mating, only one female did not ovulate in the presence of the test male. That female produced a viable clutch with another male. Two males had

two successive matings with only a day between them, and in both cases, the egg division rate in the clutches was $\geq 99\%$.

The male with the poorest reproductive performance was the largest, at 204 mm CL; that male did not fertilize the second and fourth females. The other males with reproductive failures were 140 and 163 mm CL (Table 1).

DISCUSSION

In this study, we wanted to duplicate the natural male reproductive condition during the short mass multiparous crab molting season. Primiparous red king crab may molt earlier than multiparous females (Stone et al. 1992), and it is possible that some test males mated with them before capture. If this happened, then these laboratory experiments understate the total reproductive potential of some test males. However, the male harvest occurs before the mating seasons of both morphotypes, and any males available to breed multiparous females would have been on the grounds during the earlier primiparous breeding. So, this study purposefully used males that had been on the breeding grounds during the primiparous mating period as test subjects to see if they could breed three females in 20 days, as predicted by Zheng et al. (1995).

In 10 of the matings, the males had copulated the previous day. All but one of them fertilized $\geq 86\%$ of the eggs in their mate's clutch, suggesting that most males ≥ 140 mm CL do not need a long rejuvenation period to produce sperm for an additional mating. This is consistent with field observations of male reproductive tracts, which showed that sperm begins to accumulate after spawning and reaches maximum quantities during March of the next year, forming a reserve that is depleted during the breeding season (Sapelkin and Fedoseev 1986).

In nature, males in grasping pairs are typically >120 mm CL (Powell and Nickerson 1965, Powell et al. 1972, Powell et al. 1974). Although the size at maturity is ≈ 80 mm (Paul and Paul 1990), small mature males have not been observed in grasping pairs (Powell et al. 1972, Powell et al. 1974). Sublegal-sized males cannot breed as many females (Paul and Paul 1990) as do large males. Red king crab males 80–89 mm CL were successful in inducing ovulation with 75, 38, 12, and 12% of their first, second, third, and fourth potential mates, respectively. In red king crab, larger males have bigger and more numerous spermatophores and

TABLE 1.
Percentage of eggs dividing in clutches of *P. camtschaticus* mated successively by a single male.

Male CL\Chela Height (mm)	Mate 1 % Eggs Dividing	Days Between Mating	Mate 2 % Eggs Dividing	Days Between Mating	Mate 3 % Eggs Dividing	Days Between Mating	Mate 4 % Eggs Dividing
140\42	100	22	99	3	100	1	Another male
140\42	99	9	98	4	99	No female	
145\44	100	14	99	3	100	4	95
163\50	99	1	86	2	5	7	0*
176\53	99	14	99	1	99	1	99
204\62	99	6	Another male	1	99	14	Another male
189\61	100	1	100	5	100	6	79
190\59	100	6	100	6	100	1	99
192\61	97	2	98	1	98	2	98
199\64	100	2	100	1	100	1	100

The crabs (both sexes) from the first six experiments were from Cook Inlet, and the last four were from Kodiak, AK. A note of Another male indicated male did not induce female to ovulate and she had to be bred by another male. A 0* indicated a clutch with only nondividing eggs. Blank spaces indicate no female was available.

more of them than do sublegal-sized males (Sapelkin and Fedoseev 1986, Paul et al. 1991). There is also considerable variability in the amount of sperm carried by males, which may be related to the number of females they have bred (Sapelkin and Fedoseev 1986); this may explain why not all males fertilized a fourth mate. Previous experiments showed that males can mate with seven or more females (Powell et al. 1974), but when ≥ 140 mm CL males had to breed four females within 22 days or less, the fourth successive female was not fertilized in 33% of the cases (Table 1). The reason for this reproductive failure is unknown because there is no information on the amount of sperm stored by the test males, how much sperm is normally used during the breeding season, how quickly males replenish sperm after mating, or if large males replenish sperm stores faster than small ones. Although a male's full reproductive capacity may be approximately seven mates (Powell

et al. 1974), our study suggests that it may be more realistic to expect legal-sized males to fertilize no more than three multiparous females for purposes of population modeling like that of Zheng et al. (1995).

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MOLT TIMING AND GROWTH OF THE LOBSTER, *HOMARUS AMERICANUS*, OFF NORTHEASTERN CAPE BRETON ISLAND, NOVA SCOTIA

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ABSTRACT Seasonal changes in molt condition, together with mark-recapture data, were used to estimate molt timing and growth of adolescent and adult lobsters (*Homarus americanus*) in the St. Anns Bay area (northeastern Cape Breton Island) in 1993 and 1994. In both years, most molting occurred between late July and early September. Within the larger sizes (>70 mm CL), males molted earlier than females, but there was extensive overlap in their molting periods. Annual differences in molting time were apparent and were linked to bottom temperature. Double molting of individual lobsters was rare and limited mainly to prerecruit lobsters. From spring to autumn, there were substantial changes in trap catch rate, size composition, and sex ratio. Males dominated the catch in late summer and fall, probably because they molted earlier than females. Within-season and between-year changes in catchability associated with molting need accounting if trap catch rates are to be used as indices of lobster abundance and for estimation of exploitation rate.

KEY WORDS: molting, *Homarus*, temperature, catchability, Nova Scotia

INTRODUCTION

The waters off northeastern (NE) Cape Breton Island, in northern Nova Scotia (Fig. 1), have yielded high lobster landings per square kilometer relative to other areas of coastal Nova Scotia (Hudon 1994). Estimates of molt increment and molt probability are unavailable for this area and are needed for population and management models. Molt probability of sublegal-sized and legal-sized lobsters in NE Cape Breton is of particular interest because in the adjacent southern Gulf of St. Lawrence, two annual molts can occur (Templeman 1936, Munro and Theriault 1983), whereas along the Atlantic Coast of Nova Scotia, single annual molts are the rule (Robinson 1979). Given the link between growth and reproduction in *Crustacea*, lobsters in NE Cape Breton might be expected to have growth patterns more similar to those of the southern Gulf of St. Lawrence, because of their similar sizes of maturity. The size at which 50% of females are mature is estimated to be 70–80 mm carapace length (CL) in the southern Gulf of St. Lawrence, about 73 mm CL in NE Cape Breton, and 80–95 mm CL along the Atlantic Coast of Nova Scotia (Watson 1988, Pezack and Maguire 1995).

Factors that can affect molt probability in lobster (*Homarus americanus*) include temperature, season, photoperiod, density, habitat, nutrition, and social interactions (Hartnoll 1982, Waddy and Aiken 1995). Temperature is particularly important, and there are consistent geographic differences in the number of annual molts that may be related to seasonal temperature regimes. Templeman (1936) noted that lobster molting in the Canadian Maritimes could occur as early as June in areas with high early summer temperatures, such as the southern Gulf of St. Lawrence. A second molting period was possible in late summer or early fall. Two molting periods can also occur in Long Island Sound (Stewart 1972), although in some years, molting activity peaks in June and then continues at a low level until December, with no distinct second peak (Keser et al. 1983). Whether there are two distinct molting periods or a single protracted period, data are usually lacking on whether individual lobsters molt twice in one year ("double molt").

During the 1980s, temperature in the St. Anns Bay area (Fig. 1) was intermediate to that in areas with two molting periods and those with one (Fig. 2). This suggests the potential for two molting periods in St. Anns Bay, and perhaps double molts for some individual lobsters. In this article, we evaluate the timing and number of molting periods for adolescent and adult lobsters (sensu Lawton and Lavalli 1995) off NE Cape Breton in two consecutive years. We also estimate growth increments and molt probability. Our approach to these questions is a combination of biweekly trap sampling of the lobster population and a series of mark-recapture experiments.

MATERIALS AND METHODS

Temperature

Bottom temperature was measured to assess any relationship it might have with molt timing. Recorders (Ryan J and Hobo-Temps) were deployed in traps by one or two commercial fishermen during the May 15 to July 15 fishery and were anchored to the bottom after the fishery closed. Shallow (4–8 m) and deep (16–20 m) depths were assessed, but the deep recorder deployed from July 20 to mid-September 1993 was unrecoverable. Measurements were made off Little River except for mid-May through mid-July 1993, when temperatures at 4–8 m were recorded off Englishtown (Fig. 1). Degree days were calculated as the cumulative sum of daily average temperatures at the shallow depth. Data were interpolated for the gap in the temperature record in the second half of July of both years.

Molt Indices From Trapping

Lobsters off the fishing port of Little River (Fig. 1) were sampled approximately every 2 wk from late May until late September in 1993 and 1994. Lobsters were obtained by commercial traps during the spring (May 15 to July 15) and by research traps thereafter. Commercial traps numbered 200–275 per date and were set over about 8 km of coastline; research traps numbered 50–100 and were set over 4 km within the same section of coastline. Traps

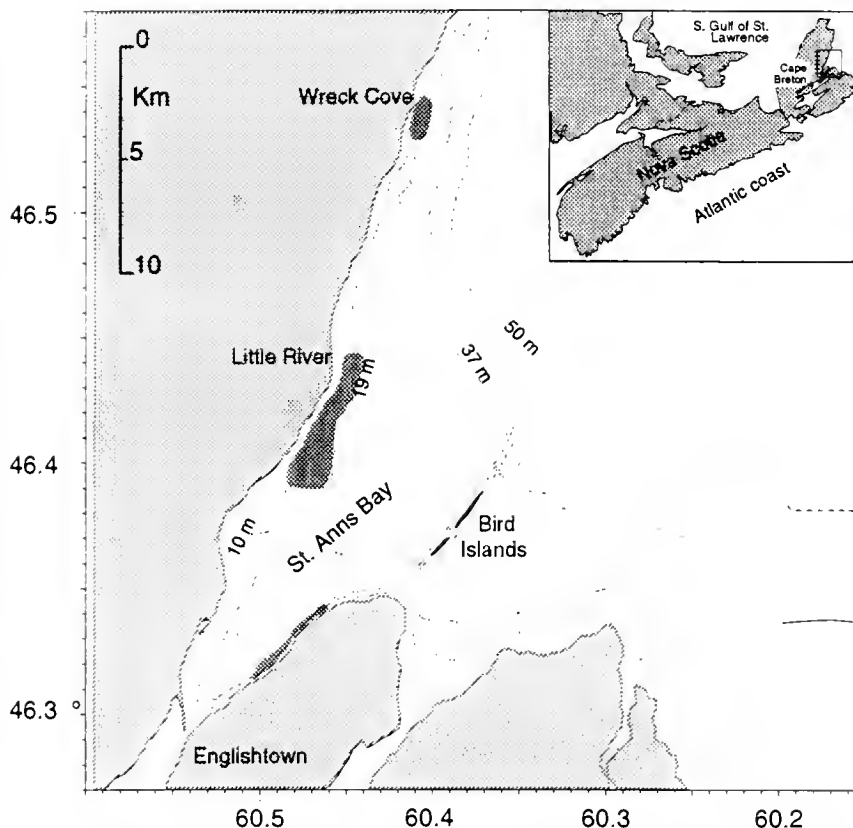


Figure 1. St. Anns Bay and adjacent area, with inset of the Canadian Maritimes. Shaded areas show where seasonal trapping for molt staging was conducted (off Little River) and where lobsters were tagged (off Little River, in Wreck Cove, and north of Englishtown).

were set at depths of 3–25 m, with most traps set between 10 and 20 m. Commercial traps (variable sizes) were of the traditional wood lath design with two 13-cm ring entrances into a baited compartment and a funnel into a second compartment. These traps had 38 by 150 mm slot openings to allow the escape of sublegal lobster (<70 mm CL in this management area). The research traps (90 × 47 × 35 cm) also had two compartments with two 13-cm circular entrances. They were constructed of plastic-coated wire with mesh squares of 3.5 by 3.5 cm. Because there were no escape slots, the research traps were expected to retain more sublegal-sized lobsters than were the commercial traps. Both trap types were baited with mackerel or herring and were set overnight, usually for 24 h.

Shell condition was used as one measure of molting date. Lobsters were classified as postmolt if the shell had bright colors and a shiny surface with few abrasions, and if the lateral portion of the posterior carapace could be depressed with light finger pressure ("soft" or "buckle-shelled"). These characteristics suggest that molting occurred within the previous 1–5 wk (Donahue 1954, Aiken 1980).

Pleopods were molt staged as another measure of molt timing. Beginning in early June of both years, pleopods were removed, stored in ambient seawater (on ice in August and September), and staged according to Aiken (1973) within 8 h of collection. Pleopods were grouped on the basis of lobster CL: 55–70 and 70–93 mm. An upper limit of 93 mm CL was chosen because it is approximately two molts beyond legal size, and because few lobsters beyond this size were available. Pleopods were not staged if the lobster had clearly molted recently, or if it was ovigerous (to avoid

egg damage). Pleopod stages were grouped as follows: 0–2.5, 3.0–3.5, and ≥ 4.0 . These three groups correspond to molt stages C_4 – D_0 , D_1 '– D_1 ", and D_1 "– D_3 (Aiken 1973). After pleopod stage 3.0, no developmental plateaus occur, and time to ecdysis is determined primarily by temperature (Aiken 1973). The number of pleopods staged per date for each of the four size/sex groups averaged 28.6 in 1993 (range, 4–93) and 27.6 in 1994 (range,

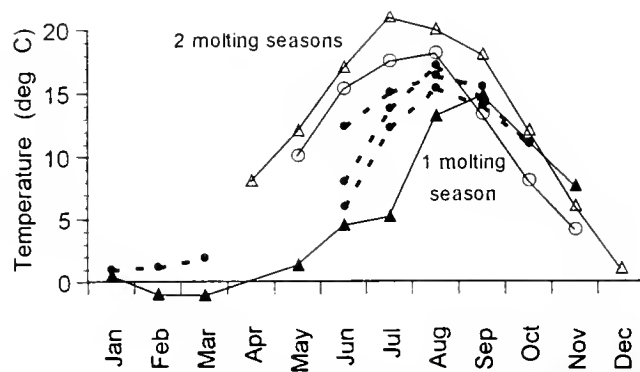


Figure 2. Mean monthly bottom temperature in areas with one or two molting seasons and in the St. Anns Bay area. Areas represented are: Malpeque Bay 1983 (Δ); off Magdalen Islands 1978 to 1979 (\circ), off Englishtown and in Wreck Cove in 1983 and 1986 to 1987 (dashed line), and off New Harbour 1985 (\blacktriangle). Temperatures were measured at depths of 3–11 m. Data sources: Moriyasu (1984), Munro and Theriault (1983), and unpublished Department of Fisheries and Oceans (DFO) thermograph records.

TABLE 1.

Number and size of lobsters tagged in the St. Anns Bay area (Wreck Cove-Englishtown) from July 1993 to September 1994.

Tagging Period	Males			Females			Ovigerous Females			Total Tagged
	n	MCL	SD	n	MCL	SD	n	MCL	SD	
July 21, 1993	90	68.4	9.6	73	65.9	5.3	16	78.9	5.4	179
Sept. 21–30 1993	888	80.1	10.9	443	72.3	7.3	34	79.5	7.3	1,365
May 18, 1994	54	65.4	3.1	71	65.4	3.9	13	78	5.3	138
Sept. 21, 1994	275	84.4	11.0	123	77.1	4.9	0			398
Total	1,307			710			63			2,080

MCL, mean CL (mm). SD, standard deviation of CL.

1–61). When the number of pleopods for a size/sex group was less than 5 (three cases), the molt stage distribution was interpolated.

The catch rate of the different size/sex groups (number per trap haul or $NPTH_i$) was simply the total number caught per group divided by the number of traps hauled. To estimate $NPTH$ for lobsters in a particular pleopod stage, the catch of postmolt lobsters needed to be accounted for, because their pleopods were not removed. An example calculation for male lobsters <70 mm CL in pleopod stage j ($NPTH_{ij}$) is as follows:

$$NPTH_{ij} = NPTH_i \times P_{ij} \times (1 - SS_i)$$

where $NPTH_i$ is the number of males <70 mm CL per trap haul, P_{ij} is the proportion of males <70 mm CL in pleopod stage j , and SS_i is the portion of males <70 mm CL in the catch that are postmolt.

The weighted mean calendar date (MCD) of occurrence of a particular molt stage was calculated as:

$$MCD = \sum_{i=1}^n CD_i \cdot NPTH_i / \sum_{i=1}^n NPTH_i$$

where CD_i is the consecutive day in the year on sampling day i , $NPTH_i$ is the number per trap haul on sampling day i , and n is the number of sampling dates.

Molt Incidence From Mark-Recapture

A total of 2,080 lobsters were tagged in the St. Anns Bay area between July 1993 and September 1994 (Table 1). Most lobsters (1,640) were tagged off Little River; 218 lobsters were tagged in Wreck Cove, and 222 were tagged on the eastern side of St. Anns Bay, just north of Englishtown (Fig. 1, Table 1). Lobsters to be tagged were captured by research traps or commercial traps. Usually, all sizes and sexes were tagged, except in May 1994, when only sublegal-sized lobsters were tagged, and in September 1993, when there was some selection for females and undersized lobsters. Tag type was the polyethylene streamer type, which can yield higher tag returns than the sphyrion tag, possibly because of greater tag retention through the molt (Moriyasu et al. 1995). To insert the tags, lobsters were held with the abdomen flexed to expose the dorsal musculature, and the disposable needle was threaded through the membrane into the right abdominal muscle, up over the dorsal artery and down through the left dorsal muscle to exit on the other side. In this way, the tag was visible on both sides of the lobster. Large lobsters (greater than about 90 mm CL) were tagged only in the right dorsal muscle.

Lobsters were recaptured during the commercial fishing season (May 15 to July 15), with the exception of a few recaptures (<1%) during experimental trapping in August and September. Fishermen were involved in the tagging and were informed of the need to measure the CL of the lobsters before removing the tag. As an incentive, fishermen received \$3.00 for tag information. Most measurements (about 80%) were made to the nearest millimeter by trained technicians or by the authors; about 20% were made by fishermen. In some cases, fishermen indicated the lobster grade ("canner" or "market"), which sometimes enabled us to discern whether a lobster molted, but not the size of the growth increment. In 1994, many of the lobsters were returned to the bottom after capture and measurement; some of these were captured again in later years, and if growth information was recorded, it is included in the analysis.

The relationship between growth increment and size was analyzed by regressing molt increment on premolt size rather than the often-used approach of regressing postmolt size on premolt size. Using the latter approach, variation among areas or times is diffi-

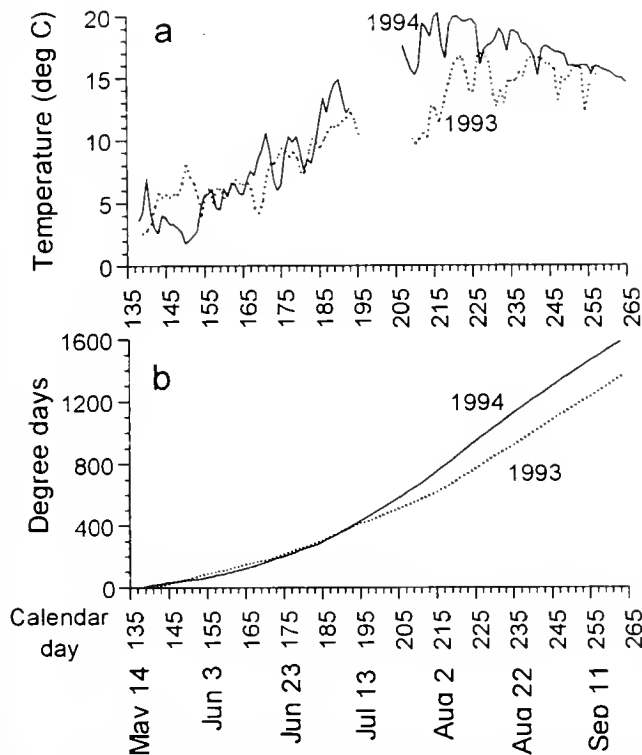


Figure 3. Seasonal temperatures (a) and degree days (b) in St. Anns Bay in 1993 and 1994. Temperatures were measured at 4–8 m off Little River, with the exception of the May to July 1993 record (4–8 m off Englishtown).

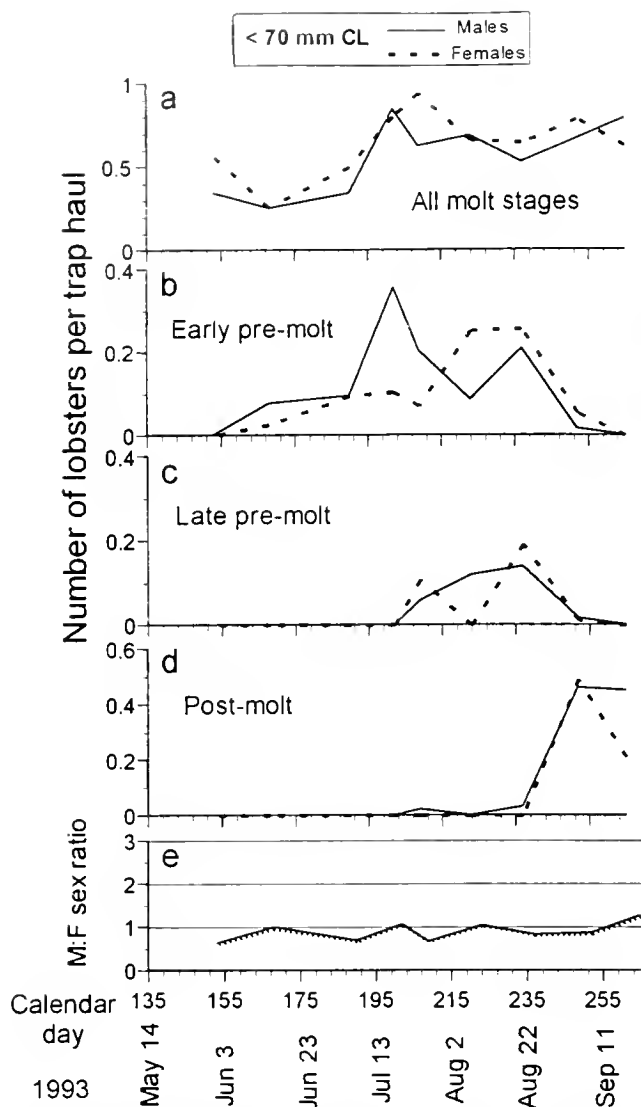


Figure 4. Catch rate and sex ratio of lobsters <70 mm CL in 1993. (a) All molt stages; (b) early premolt (pleopod stages 3.0–3.5); (c) late premolt (pleopod stages ≥ 4.0); (d) postmolt; (e) sex ratio (all molt stages). Ovigerous females are excluded in Panels a to d. Solid line in Panel e is without ovigerous females; dotted line is sex ratio with ovigerous females.

cult to detect because of the large effect that premolt size has on postmolt size (Fogarty 1995). The relationship between molt probability and size was modeled using the "anniversary" method of Hancock and Edwards (1967) applied to the data from recaptured lobsters. A logistic equation was fit to the observed data because it constrains the probability values to between 0 and 1 (Fogarty 1995). We do not have good estimates of tag loss at molt for the streamer tag; we therefore used values of 0, 10, and 25% and corrected the molt probabilities using the method of Fogarty and Idoine (1988).

RESULTS

Temperature

From mid-May to mid-July 1993, the temperature at 4–8 m bottom depth rose from 3 to 12°C (Fig. 3a). Temperatures from

late July through September reached a high of 17°C, averaging 15.1°C in August. In 1994, temperatures from mid-May until mid-July were similar to those in 1993, but late July through September temperatures were higher, reaching 20°C and averaging 18.3°C in August. Degree days from May 18 to September 21 at shallow depths reached 1,602 in 1994, compared with 1,385 in 1993 (Fig. 3b). The deep (16–20 m) temperature record is incomplete, but temperatures were generally 1–2°C lower than those at shallow depths. Infrequently, after periods of upwelling induced by southerly winds, bottom temperatures at the deep location could be 5–10°C below that of the shallow location.

Molt Indices From Trapping

Changes in catch rate of lobsters at different molt stages and in size distribution indicate that molt timing was influenced by size and sex. Annual differences in molt timing are also evident.

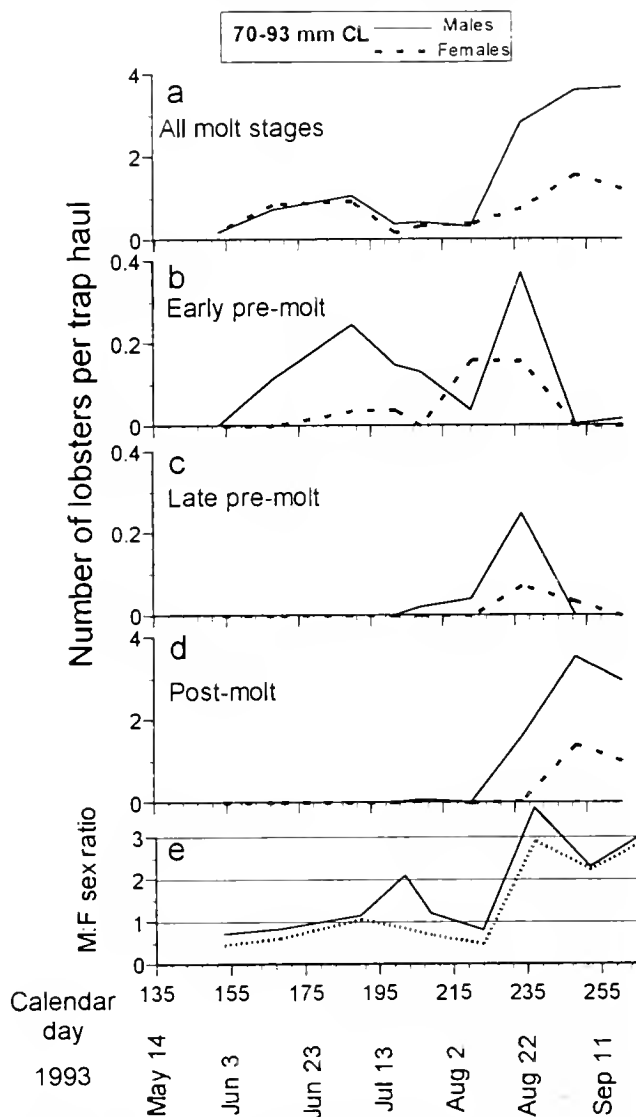


Figure 5. Catch rate and sex ratio of lobsters 70–93 mm CL in 1993. (a) All molt stages; (b) early premolt (pleopod stages 3.0–3.5); (c) late premolt (pleopod stages ≥ 4.0); (d) postmolt; (e) sex ratio (all molt stages). Ovigerous females are excluded in Panels a to d. Solid line in Panel e is without ovigerous females; dotted line is sex ratio with ovigerous females.

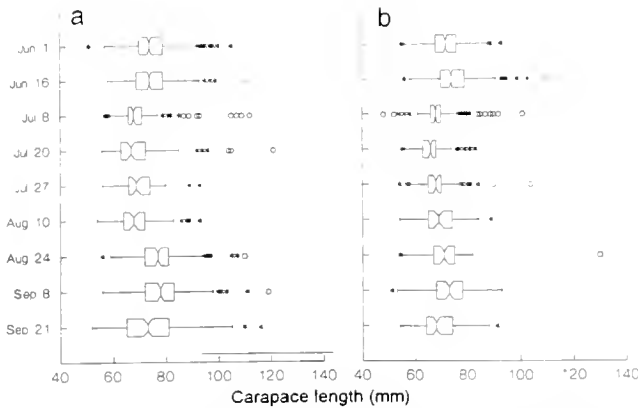


Figure 6. Notched box and whisker plots for length of lobsters collected by traps in 1993: (a) males, (b) females. Boxes are notched at medians and return to full width at lower and upper 95% confidence intervals. Sides of box correspond to quartiles; points beyond the "whiskers" are greater than $1.5 \times$ the interquartile range. Asterisks mark outside values; circles indicate far outside values (see Wilkinson et al. 1992).

1993

The catch rate of sublegal sizes (<70 mm CL) increased after July 15 in 1993 (Fig. 4a), probably because of the change to research traps with no escape gaps. The subsequent catch rate of these sizes showed no marked trend. Early premolt stages entered traps from mid-June until late August (Fig. 4b); late premolt stages entered from mid-July through early September (Fig. 4c). The catch rate of postmolt lobsters <70 mm CL rose to a sharp peak in early September (Fig. 4d). The sex ratio of sublegal-sized lobsters (Fig. 4e) was significantly different from 1:1 only on June 1 and July 8, when females were favored (χ^2 test on each date, $p < 0.05$, $df = 1$).

The catch rate of larger males and females was lowest after the fishery closed (July 15), reflecting removals (Fig. 5a). In late August, overall catch rate increased substantially, particularly for the males. Early premolt males appeared in mid-June; early premolt females appeared in late June (Fig. 5b). Late premolt and postmolt males 70–93 mm CL also appeared earlier than females (Fig. 5c and d). The sex ratio of larger lobsters was significantly different from 1:1 on June 14, July 20, and August 25 through Sept. 21 (χ^2 tests, $p < 0.05$, $df = 1$). Males were favored on all dates except for June 14 (Fig. 5e).

Significant differences in median CL are illustrated by non-overlapping confidence intervals in box plots (Fig. 6). Males shifted to smaller sizes between June 16 and July 8, reflecting fishery removals of legal sizes (Fig. 6a). Between August 10 and 24, there was a significant increase in median size (about 8 mm), reflecting the molt, whereas on September 21, there was a significant decrease in size of unknown cause. The size of females also declined significantly between June 16 and July 8 (Fig. 6b). After this date, there was no single large increase, but compared with July 20, median size was significantly greater on September 8. As for the males, there was a significant decrease in median size on September 21.

1994

As in 1993, the catch rate of sublegal sizes (<70 mm CL) increased after July 15, coincident with the change to research

traps with no escape gaps (Fig. 7a). Early premolt stages entered traps from June 23 until late August (Fig. 7b); late premolt stages entered from early July through mid-September (Fig. 7c). The NPTH of postmolt lobsters peaked on August 25 (Fig. 7d), 2 wk earlier than in 1993. The sex ratio of sublegal-sized lobsters in 1994 (Fig. 7e) was not significantly different from 1:1 on any date (χ^2 tests, $p > 0.05$, $df = 1$).

The catch rate of males and females 70–93 mm CL was again lowest after the fishery, reflecting removals (Fig. 8a). Early and late premolt stages of males appeared before females in the comparable stages (Fig. 8b and c). Postmolt males first appeared on August 10 but peaked on August 25 (Fig. 8d). Males began to dominate the catch earlier in August 1994 than in 1993, and the sex ratio again reached about 3:1 (Fig. 8e). The sex ratio (Fig. 8e) was

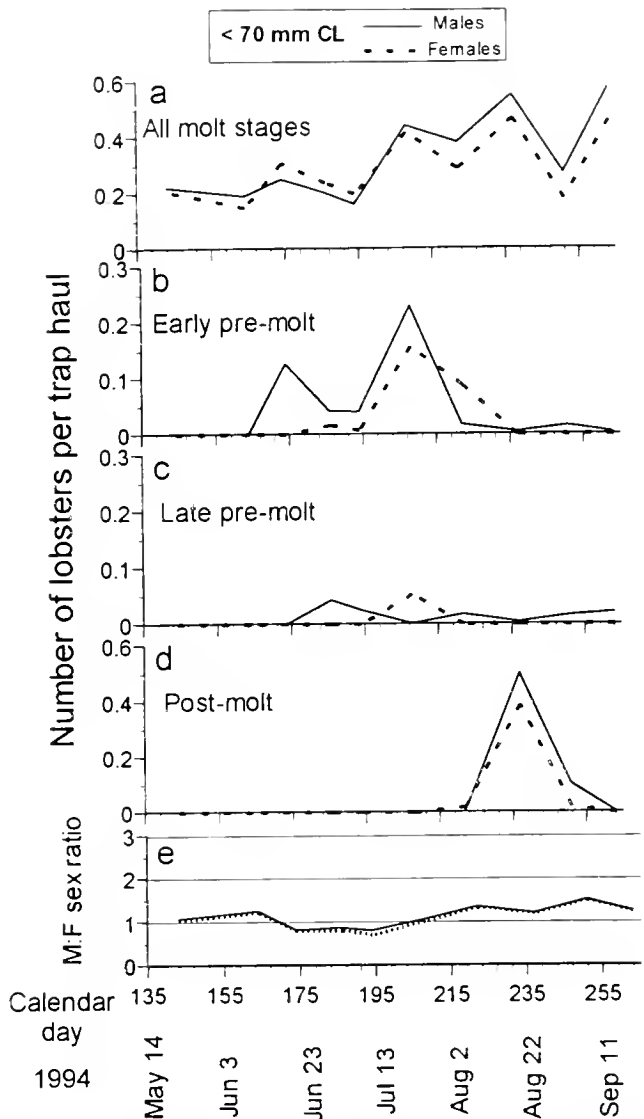


Figure 7. Catch rate and sex ratio of lobsters <70 mm CL in 1994. (a) All molt stages; (b) early premolt (pleopod stages 3.0–3.5); (c) late premolt (pleopod stages ≥ 4.0); (d) postmolt; (e) sex ratio (all molt stages). Ovigerous females are excluded in Panels a to d. Solid line in Panel e is without ovigerous females; dotted line is sex ratio with ovigerous females.

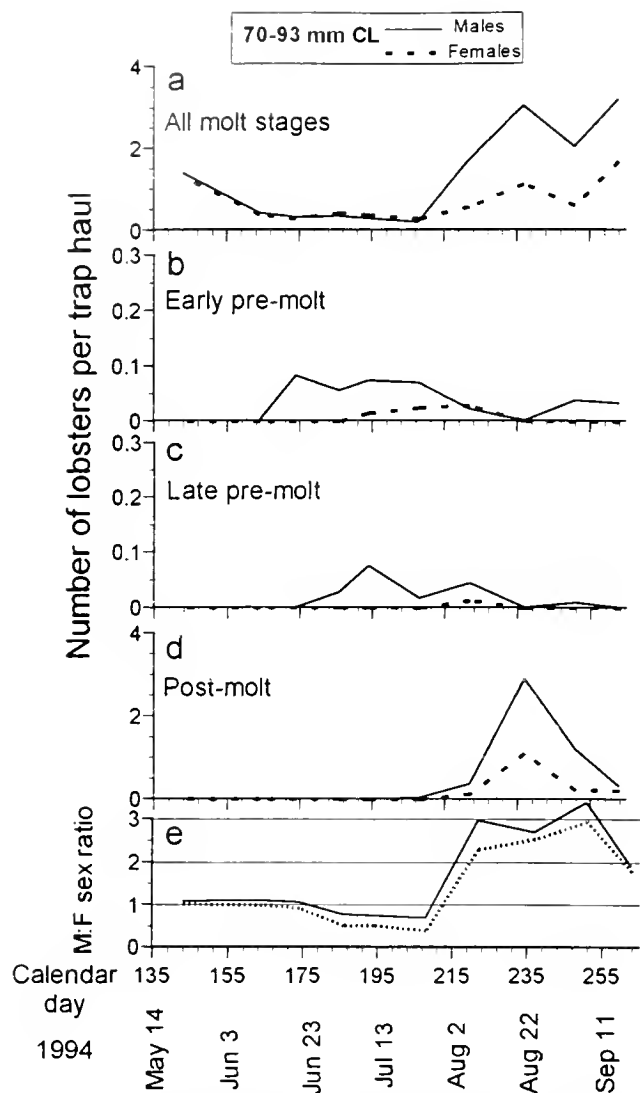


Figure 8. Catch rate and sex ratio of lobsters 70–93 mm CL in 1994. (a) All molt stages; (b) early premolt (pleopod stages 3.0–3.5); (c) late premolt (pleopod stages ≥ 4.0); (d) postmolt; (e) sex ratio (all molt stages). Ovigerous females are excluded in Panels a to d. Solid line in Panel e is without ovigerous females; dotted line is sex ratio with ovigerous females.

significantly different from 1:1 from August 10 onward, with males favored on each date (χ^2 tests, $p < 0.05$, $df = 1$). Females were more abundant in traps in early July (sex ratio of 0.7 excluding ovigerous females), but the χ^2 test was not significant. The sample size ($n = 28$) on this date was the smallest of the 2-y study, and thus, the χ^2 test lacked power.

Male median size decreased significantly between May 23 and June 23 and then shifted toward larger sizes on the following two sampling dates (Fig. 9a). On July 27, the median size was significantly lower, reflecting the shift to research traps. The size was significantly larger 2 wk later, as newly molted lobsters began to enter the catch (Fig. 9a). The size distribution on the subsequent four sampling dates was stable. The size distribution of females changed similarly (Fig. 9b). As in 1993, the size increase in August was not as distinct in the females as in the males.

Effect of Gender and Year

The weighted MCD for the catch rate of lobsters in the various molt stages in 1993 and 1994 indicate that large females were not as advanced in most molt stages and that the molt of all lobsters was earlier in 1994 than in 1993 (Table 2). To test whether there were significant differences in molt timing due to gender and year, the dates in Table 2 were converted to calendar days, and paired tests were run (Wilcoxon ranked sign) on the basis of the three molt indices (early premolt, late premolt, and postmolt). There was no effect of gender when both size groups were used (both years combined, $n = 12$, $p = 0.22$), but when only the large sizes were used, males had a significantly earlier molt date than females ($n = 6$, $p = 0.04$). The effect of year was significant, with molting occurring earlier in 1994 than in 1993 (both sizes and genders, $n = 12$, $p = 0.01$).

Molt Incidence and Growth Increments From Mark-Recapture

Of the 2,080 lobsters tagged, 1,369 were returned with the size at recapture (Table 3). Lobsters that molted were grouped by tag and recovery period and with respect to the number of summer periods they were at large, because summer (late July through mid-September) is when most molting is thought to occur (Table 3). Lobsters that molted were at large for 0, 1, 2, or 3 summer periods; those few that molted and were not at large during summer (0 summer periods) must have molted during the autumn after tagging or early the following spring. Of the approximately 458 returned females that were barren when tagged, only 2 had eggs. This is likely because those that were mature at tagging would extrude eggs after the spring fishery, and because of a lower reporting rate for ovigerous females (which cannot be legally retained).

A histogram of the frequency of growth increments (the difference between measurements at tagging and at recapture) shows some negative growth (Fig. 10), and we used these as an indicator of measurement error. There were three cases of negative growth

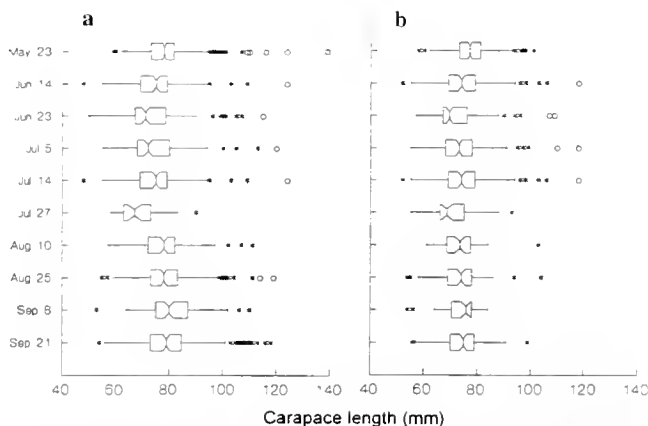


Figure 9. Notched box and whisker plots for length of lobsters collected by traps in 1994; (a) males, (b) females. Boxes are notched at medians and return to full width at lower and upper 95% confidence intervals. Sides of box correspond to quartiles; points beyond the "whiskers" are greater than $1.5 \times$ the interquartile range. Asterisks mark outside values; circles indicate far outside values (see Wilkinson et al. 1992).

TABLE 2.
Weighted mean date of occurrence (MCD) of premolt and postmolt lobsters.

Sex/Size	1993			1994		
	Early Premolt	Late Premolt	Postmolt	Early Premolt	Late Premolt	Postmolt
Males <70 mm CL	27 July	14 Aug.	12 Sept.	15 July	1 Aug.	26 Aug.
Females <70 mm CL	16 July	14 Aug.	11 Sept.	18 July	26 July	24 Aug.
Males 70–93 mm CL	14 July	20 Aug.	9 Sept.	25 June	22 July	27 Aug.
Females 70–93 mm CL	23 July	28 Aug.	12 Sept.	31 July	9 Aug.	27 Aug.

of 3 mm and two cases of positive growth of 3 mm; we attributed these to error and assumed that increments of 3 mm and less were 0 and that no molting occurred.

Whether lobsters were released in May or September, if captured before being at large for one summer, few molted (Fig. 11). Most (94–98%) lobsters <93 mm CL molted after one summer at large, but only 40% of males >93 mm CL molted after this time (Fig. 11). Even after two summers at large, not all large males had molted, but the sample size was small ($n = 5$). All ovigerous females molted after one summer at large (not shown).

The molt probability by size group after one summer at large (all tagging periods combined) shows the decrease in molt probability with size for both males and females (Fig. 12). Molt probability declined at a smaller size in females (80–85 mm CL) than males (85–90 mm CL), but the number of observations for females was low. A logistic curve provides a good fit to the observed data for males (Fig. 12a). Assuming a 25% tag loss at molt shifted the curve only a little to the right.

Growth increments for those lobsters that molted were dependent on time at large, sex, and size. When grouped by tag and recovery periods, males at large for one summer period had average growth increments of 11.2–14.5 mm (Table 4). Average increments for females in this group ranged from 8.5 to 11.7 mm. Ovigerous females had growth increments of 9.0–9.9 mm. For males at large for two molting periods, the average increments ranged from 17.1 to 29.5 mm; for females, the average increment

ranged from 15.1 to 18.0 (Table 4). Statistical comparisons of increment size among the different tag/recovery periods were not made because of the differences in sample size.

Linear regressions of growth increment on size were run for lobsters at large for less than two summer periods. Analyses were run for data groups in Table 4 with sample sizes greater than 20. There was a significant effect of size on growth increment for males tagged in September 1993 and recovered in 1994 to 1995 ($n = 181$, $p = 0.01$). There was no relationship for males tagged in July 1993 and May 1994, probably because of the narrow size range of tagged animals (60–81 mm CL in July and 60–69 mm CL in May). When data from all tagging periods were combined, the regression was highly significant ($n = 247$, $p < 0.001$; Fig. 13a). There was considerable scatter, as well as four outliers well above the regression line (Fig. 13a). These outliers may correspond to lobsters that molted twice; without them, the correlation coefficient increased from 0.30 to 0.37 ($n = 243$, $p < 0.001$).

For females, the slope of the regression was usually negative, suggesting an inverse relationship between CL and growth increment. Although significant for lobsters tagged in September 1993 ($n = 88$, $p = 0.05$), and the combined data ($n = 150$, $p = 0.02$), the relationship is doubtful because of the undue influence of three outliers (Fig. 13b). These outliers correspond to small females with relatively large increments that may have molted twice. Without them, the regressions are not significant for the two aforementioned data sets ($n = 86$, $p = 0.69$; $n = 147$, $p = 0.31$). Growth

TABLE 3.

Tag and recovery periods for lobsters tagged in St. Anns Bay area (Wreck Cove-Englishtown) from July 1993 to September 1994.

Tag Period	Recovery Period	Time at Large		n Returns With Size at Recapture
		Months	No. of Summers	
July 1993	May 15–July 15 1994	10–12	1	61
	May 15–July 15 1995	22–24	2	30
	May 15–July 15 1996	34–36	3	4
Sept. 1993	May 15–July 15 1994	8–10	0	546
	Aug. 10 1994–July 15 1995	11–22	1	293
	May 15–July 15 1996	32–34	2	45
May 1994	May 20–July 15 1994	0–2	0	44
	May 15–July 15 1995	12–14	1	67
	May 15–July 15 1996	24–26	2	6
Sept. 1994	May 15–July 15 1995	8–10	0	252
	May 15–July 15 1996	20–22	1	21
				Total 1,369

Number of summers is an indicator of the number of annual molting periods (late July to mid-September) that lobsters were at large. Some lobsters were returned to the sea after initial measurements and were recaptured at a different size in later years.

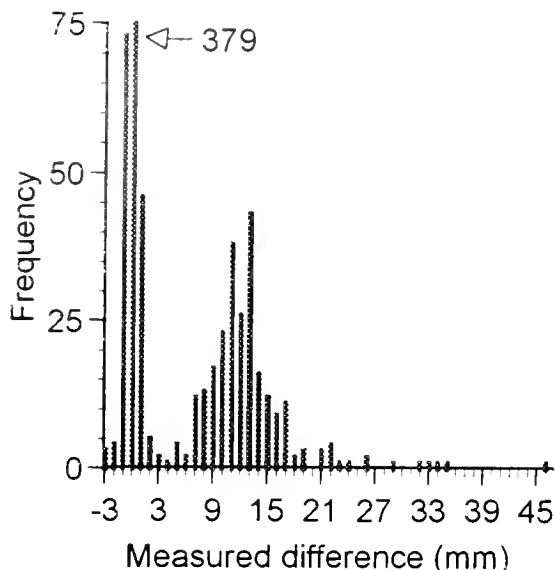


Figure 10. Frequency of measured increments between time of tagging and time of recapture for lobsters tagged in 1993. In 379 cases, there was no change in CL. Increments of 3 mm and less were attributed to measurement error.

increments in ovigerous females (Fig. 13c) showed no relationship with size ($n = 25$, $p = 0.68$).

DISCUSSION

Molt Timing, Gender, and Implications for Catchability

Within the larger sizes (>70 mm CL), premolt males entered traps at earlier dates than females in both years. From this, we infer that mature males molted earlier than mature females, as Templeman (1934) concluded for Northumberland Strait lobsters. A difference in molt timing between mature males and females is necessary, given the dominant pattern of mating behavior. Most mating occurs between soft-shelled females and harder-shelled males (Templeman 1934, Atema et al. 1979, Waddy and Aiken 1995); thus, if mature males are to molt annually, they must molt at a

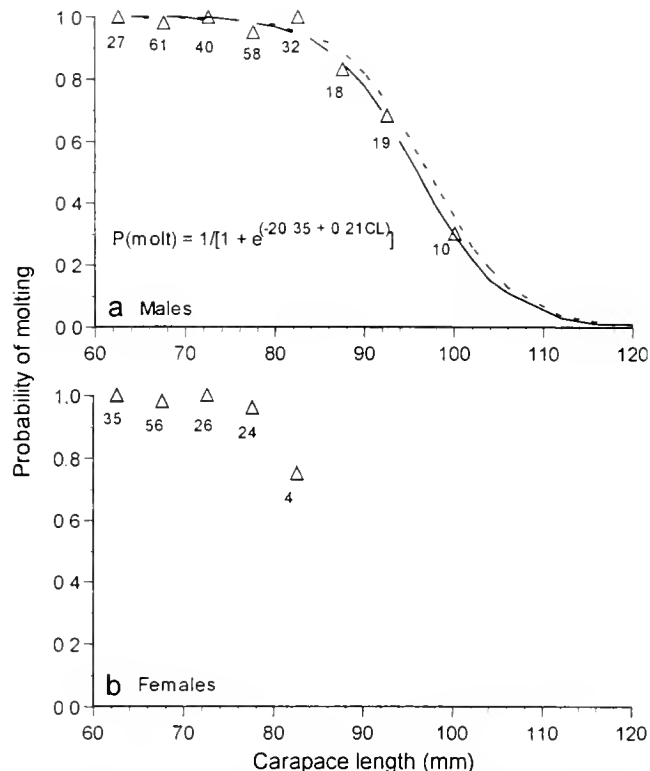


Figure 12. Molt incidence of lobsters at large for one molting period (late July through mid-September). All tagging periods are combined. Size intervals are 5 mm except for the largest male size class (95–105 mm CL). Triangles are n molted/ n returned; number of returns for each point is shown. A logistic curve has been fitted to the male data. The solid line assumes no tag loss at molt; the dashed line assumes 25% tag loss at molt.

different time than females. Females are usually mated within 12 h of molting; males on the other hand cannot mate for days to weeks after molting because they must be able to turn the female on her back and must have copulatory appendages that are hard enough for insertion (Templeman 1934).

Postmolt males and females appeared in the traps on about the same date in both years, even though premolt males entered traps before premolt females. Postmolt males greatly outnumbered females however, and probably only the earliest molting females entered traps when postmolt males first appeared. In general, premolt lobsters are less catchable in baited traps due to reduced feeding and are more catchable after molting, as the feeding rate increases (Templeman 1939, Weiss 1970, Ennis 1973, Miller 1990). For mature males, there may be a delay in their increased feeding after molting because they search for suitable shelters before mating and attend to females for up to 7 days after mating (Atema et al. 1979, Karnofsky et al. 1989). During this period, they would presumably be less likely to enter baited traps.

The differences in molt timing of males and females may explain seasonal differences in lobster catchability. The high male-to-female sex ratios during late summer and fall observed in this study have been observed elsewhere (Templeman 1939, Ennis 1980, Miller 1995). Off the West Coast of Newfoundland, Templeman (1939) could find no reason for the "comparative scarcity of female lobsters in catches." In Northumberland Strait, the same phenomenon was observed and was attributed to spatial segrega-

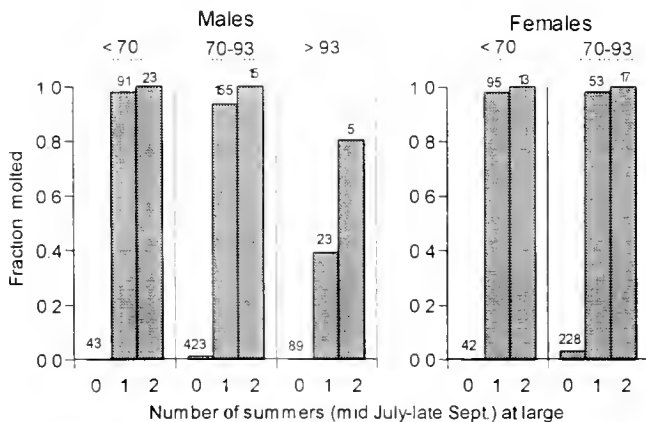


Figure 11. Molt incidence of male and female lobsters in size groups <70 mm CL, 70–93 mm CL and >93 mm CL (males only because of insufficient numbers of large females). Data for July, May, and September tagging periods were combined to increase sample size, but each tagging period showed a similar trend. Numbers returned for each category are shown at the top of the bars.

TABLE 4.
Premolt CL and growth increments for recaptured lobsters tagged in St. Anns Bay.

Tag Period	Recovery Period	n Summers	Sex	n	Premolt CL (mm)				Increment (mm)		
					Mean	SD	Min	Max	Mean	SD	%
July 1993	May–July 94	1	M	29	68.5	5.25	60	81	11.4	2.18	17
	May–July 95	2	M	22	66.3	10.00	57	104	17.1	5.99	26
	May–July 96	3	M	2	76.0	28.28	56	96	16.5	0.71	22
Sept. 1993	May–July 94	0	M	1	74.0		74	74	7.0		9
	Aug. 94–July 95	1	M	180	75.7	8.23	59	95	12.9	2.63	17
	May–July 96	2	M	16	75.7	15.94	52	104	21.8	9.07	29
May 1994	May–July 95	1	M	24	65.8	2.66	60	69	11.2	1.49	17
	May–July 96	2	M	2	64.0	1.41	63	65	29.5	9.19	46
Sept. 1994	May–July 95	0	M	3	77.0	10.44	70	89	9.0	3.00	12
	May–July 96	1	M	10	85.9	14.12	71	121	14.5	3.81	17
All	All	0–1	M	247	74.3	8.91	59	121	12.5	2.69	17
	All	2	M	40	69.3	13.23	52	104	19.6	7.98	28
July 1993	May–July 94	1	F	24	66.4	3.98	59	73	9.3	1.95	14
	May–July 95	2	F	9	62.4	3.68	57	68	17.4	5.27	28
	May–July 96	3	F	2	64.5	4.95	61	68	17.5	0.71	27
Sept. 1993	May–July 94	0	F	4	73.0	2.16	70	75	5.3	1.26	7
	Aug 94–July 95	1	F	84	70.1	5.84	54	82	10.8	1.89	15
	May–July 96	2	F	23	75.1	9.09	59	95	15.1	8.92	20
May 1994	May–July 95	1	F	34	65.4	2.78	60	69	11.7	3.31	18
	May–July 96	2	F	4	63.3	7.63	52	69	18.0	4.69	28
Sept 1994	May–July 95	0	F	2	75.0	1.41	74	76	9.5	3.54	13
	May–July 96	1	F	2	74.0	5.66	70	78	8.5	2.12	11
All	All	0,1	F	150	68.6	5.42	54	82	10.6	2.56	15
	All	2	F	30	70.6	9.84	52	95	16.0	7.32	23
July 1993	May–July 94	1	FO	5	81.0	6.20	75	91	9.4	1.95	12
Sept. 1993	Aug 94–July 95	1	FO	16	75.6	4.99	68	86	9.9	1.57	13
May 1994	May–July 95	1	FO	4	75.5	3.79	73	81	9.0	1.83	12
All	All	1	FO	25	76.7	5.35	68	91	9.7	1.65	13

n summers is an indicator of the number of annual molting periods (late July to mid-September that lobsters were at large. Main recovery period was the commercial fishing season (May 15 to July 15). FO, ovigerous female.

tion of the sexes—males dominated catches in 8–10 m, whereas females outnumbered males in depths of 15–22 m (Templeman 1939). By comparing diver surveys with trap catches, Ennis (1980) found that spatial segregation of the sexes was not the cause, because diver-determined sex ratios of lobsters >80 mm CL were much closer to 1:1. In Sydney Harbour, about 40 km southeast of the study site off Little River, Miller (1995) also compared diver-determined sex ratios with those in trap catches and found males to be much more catchable in traps than females. Observations in this study of a gradual increase in the percentage of males, coupled with the earlier appearance of premolt males, indicate that male-dominated catches in late summer are related to earlier male molting, and possibly a slower postmolt recovery by females.

Although males are more catchable than females in late summer and fall, this does not appear to be the case during spring, when the fishery occurs. In fact, the sex ratio may be skewed in favor of females in June and July (Figs. 5e and 8e) and may be related to males becoming less catchable as they prepare to molt. Seasonal differences in catchability coefficients probably explain why simulation of a fished lobster population using catchability coefficients determined in late summer do not produce the sex ratios observed in commercial lobster fisheries (Miller 1995). Seasonal differences in catchability have clear implications for the use of fishing success methods with their assumptions of constant catchability (Ricker 1975, Miller and Mohn 1993).

Annual Differences in Timing of Molt

In 1994, adolescent and adult lobsters of both sexes molted at least 2 wk earlier than in 1993. The most probable explanation for the earlier molt is the higher temperature in summer 1994 (Fig. 3). Once lobsters have entered molt stages D_0 to D_3 , time to ecdysis is correlated with ambient temperature (Aiken 1973). At a pleopod stage of 3.0, for example, lobsters held at an ambient temperature of 19°C will molt after 18–20 days; at an ambient temperature of 15°C, the same lobsters will take 26–28 days to molt. The average temperature in August 1994 was 18.3°C compared with 15.1°C in August 1993. Thus, at constant temperatures, we would expect molting to be about a week earlier in 1994. The fact that temperature in St. Anns Bay was not constant (and reached 20°C at the shallow depth) in August 1994 probably explains why lobsters molted 2 or more weeks earlier than in 1993. Correlation between temperature and molt timing has been reported elsewhere—in Long Island Sound, annual molting peaks occur as early as mid-June, when the average May bottom temperatures is warm (10.8°C), and as late as mid-July, when May temperature averages only 8.4°C (NUSCO 1995).

Incidence of Two Molts in One Year

The molting period in St. Anns Bay was protracted, occurring mainly from late July until mid-September. Any molting between

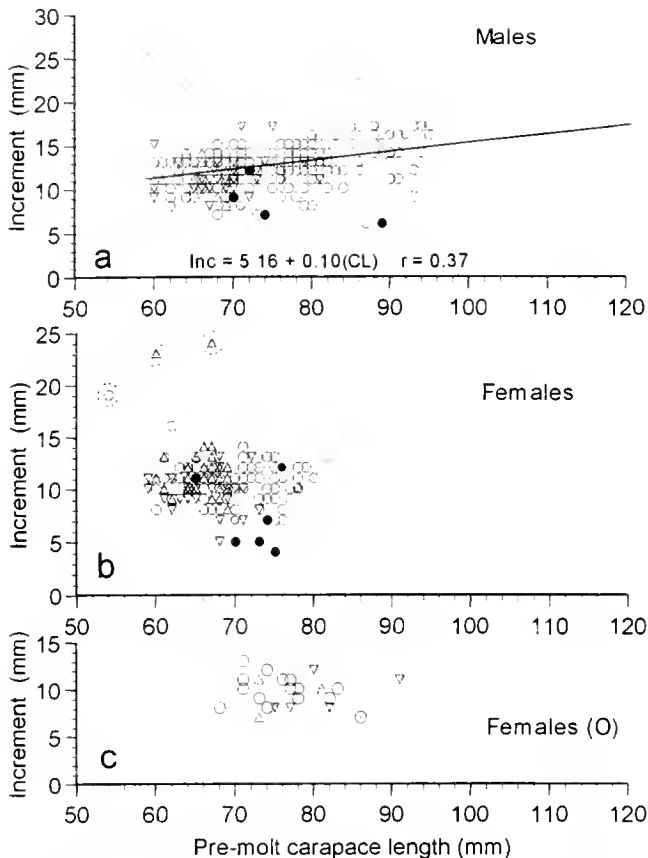


Figure 13. Molt increment versus size for lobsters that grew and were at large during up to one molting period. (a) males ($n = 247$), (b) females ($n = 150$), and (c) ovigerous females ($n = 25$). Symbols represent different tagging periods and whether lobsters were at large for part or all of one "normal" molting period (late July to mid-September), or whether they molted outside of this period. Symbols as follows: (∇) tagged in July 1993; (\odot) tagged in September 1993; (\triangle) tagged in May 1994; (\circ) tagged in September 1994; (\bullet) tagged in September 1993 or 1994, molted between tagging and recapture in the following spring. Circled outliers in Panels a and b may represent double molters. Regression line in Panel a is for all male data except outliers.

late September and June was rare, as was double molting by individuals. We would expect that lobsters that molted twice over a given period at large would have growth increments that were about double the average. For lobsters at large for one molting period (late July to mid-September), this was the case for 4 of 247 males (1.6%) and 3 of 150 females (2.0%) (outliers in Fig. 13). The four males with large increments were tagged in September 1993 and recovered 20–21 mo later, in May and June 1995. It is possible that these lobsters molted in the autumn after tagging and in autumn 1 y later, but this seems unlikely, given that only about 1% of lobsters molted between September and the following spring. The three females with large increments were tagged in May 1994 and recovered between May 23 and July 3, 1995. They were not recorded as soft shelled on recovery, and thus must have molted twice in the period between May 1994 and May 1995 or had abnormally large increments during a single molt. As might be expected, five of the seven lobsters that appear to have molted twice in 1 y were less than 65 mm.

Higher summer temperatures in consecutive years might result in more lobsters molting twice within 1 y. An earlier molt in 1 y, coupled with high summer temperatures, might induce more lobsters to enter the premolt stages in the fall of that year. These lobsters would then reach a developmental plateau in late fall and winter but would be in an advanced state of premolt the following spring. If temperatures were again high, an earlier summer molt would result, and there might be time for recovery and an additional fall molt for some lobsters. The frequency of warm years in this area is not well documented, but it is apparent that temperatures during the mid-1980s (Fig. 2) were generally lower than those during summer 1994; therefore, it is unlikely that double molting during those years was significant.

Growth Increments and Molt Probabilities

The growth increments recorded for lobsters off Little River are in line with those from other studies of *H. americanus* in the southern Gulf of St. Lawrence and along coastal Nova Scotia (Fig. 14). Variation among reported increment-size relationships is non-rivial, with estimated increments for an 80 mm CL male ranging from 10.3 (13%) to 12.9 mm (16%) in this study. Variation appears to be less for females, with estimated increments for an 80 mm CL animal ranging from 9.7 (12%) to 11.4 mm (14%). Whether the variation among areas depicted in Figure 14 is statistically significant would require reanalysis of the original data sets. Variation in growth increments has been attributed to area and annual differences in food, water temperature, and possibly genetics (Aiken 1980, Campbell 1983a). Population models that incorporate

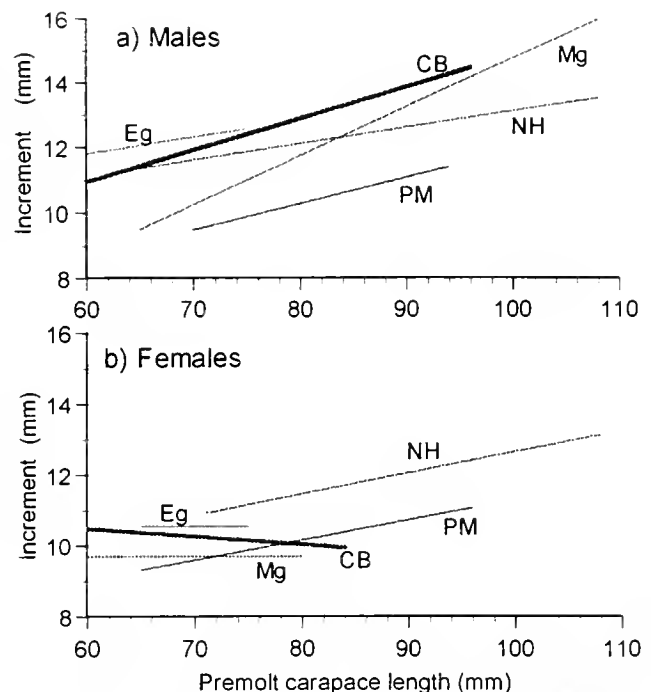


Figure 14. Regression lines for molt increment versus lobster size in the southern Gulf of St. Lawrence and coastal Nova Scotia. Egmont Bay (Eg) and Magdalen Islands (Mg) are in southern Gulf of St. Lawrence, New Harbour (NH), eastern shore of the Atlantic Coast of Nova Scotia, and Port Maitland (PM), off southwest Nova Scotia. CB = NE Cape Breton. Equations from Miller et al. (1989) and this study. Relationships are shown only over data range of original study.

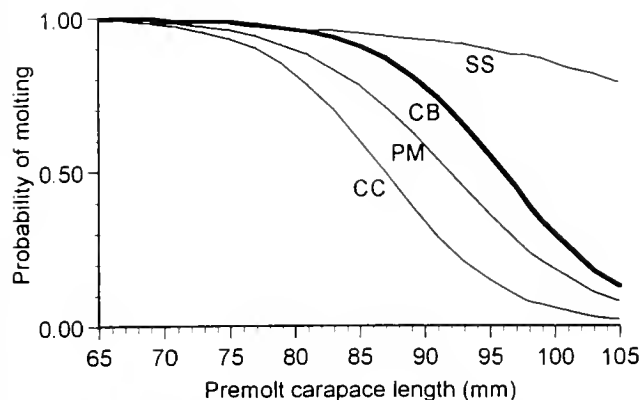


Figure 15. Molt probability and male lobster size in Comfort Cove (CC), Newfoundland; NE Cape Breton (CB, this study); Port Maitland (PM), off southwest Nova Scotia; and offshore Scotian Shelf (SS). For CC and PM, logistic curves were fit to data in Ennis et al. (1982, Fig. 8b) and Campbell (1983; 1960 to 1961 data in Table 3). SS data are from logistic equation from D. Pezzack (pers. comm.).

growth should consider the model sensitivity to variation in growth increments.

Increments increase with size for males in each study (Fig. 14a), but in just two of the five studies of females (Fig. 14b). The difference among female studies is probably related to size at maturity—in the three areas showing no increase with CL, the 50% size at maturity is 75 mm or less, whereas in the areas with increasing increments with size, the 50% size at maturity is 90–100 mm. Reduced growth increments among mature females in St. Anns Bay and in other areas (e.g., Bay of Fundy; Campbell 1983b) are expected, given their investment in reproduction (Aiken 1980). Thus, if growth increment data include reproductive-phase females, increments are unlikely to continue to increase with size.

The probability of molting for male lobsters in the St. Anns Bay area is intermediate to that of other Maritime and Newfoundland areas (Fig. 15). Only offshore lobsters on the Scotian Shelf appear to have higher molt probabilities above 85 mm. For St. Anns Bay, Newfoundland, and Port Maitland (southwest Nova Scotia), the molt probabilities for 95–105 mm CL lobsters are probably too low. Ennis et al. (1982) assessed molt probability using annual shell condition surveys and suggested that their low estimates for molt probability were related to low catchability of larger, newly molted lobsters. If larger lobsters are less catchable, then molt probabilities estimated for larger lobsters from tagging studies such as ours might also be artificially low. Data on return rates of lobsters tagged in September 1993 and 1994 are not indicative of a large effect of size on catchability. Of 71 tagged lobsters that

were >100 mm CL, a total of 39 were returned (55%), compared with a 59% return rate for the 1,360 tagged lobsters between 70 and 100 mm CL. Another potential factor that might explain low molt probabilities at larger sizes is tag loss at molt, but this is unlikely to be important, because even a 25% tag loss at molt has only a minor effect on the fitted logistic curve (Fig. 12a).

The lower molt probabilities with larger sizes might result from an effect of high exploitation on the proportion of molters within a size class (D. Pezzack pers. comm.). If lobsters above a certain size are molting every other year (probability of molting of 0.5), but the fishery is removing a significant portion of the lobsters as they enter the size class, then there will be a lower proportion of lobsters in their second year in the size class (i.e., ready to molt). Thus, if a sample is taken for tagging, the proportion molting after 1 y will be lower than 0.5. Current annual exploitation estimates by the commercial fishery off NE Cape Breton are high (65–80%; Tremblay and Eagles 1996). The effect of this could be substantial—if only 30% of the lobsters between 95 and 105 mm CL are in their second year, then our observation of 3 of 10 male molters in this size class (Fig. 12a) translates to a molt probability of 0.5 (three of a potential three molters in their second year). It is of interest that the offshore Scotian Shelf lobster fishery is thought to have a relatively low exploitation rate (15–25%; Pezzack and Duggan 1995), and estimated molt probabilities drop off much more slowly for male lobsters from this area (Fig. 15). Any effect of high exploitation rates on the estimation of molt probability from mark-recapture could be eliminated if only newly molted lobsters were tagged.

This study finds that two molts within 1 y for individual adolescent and adult lobsters off NE Cape Breton Island are rare and limited mainly to prerecruit lobsters. Estimated annual molt probability is near 1.0 for lobsters up to 80–85 mm CL and then declines. This study also demonstrates that molt timing can vary between years by at least 2 wk, probably because of annual differences in temperature. Higher catchabilities of males in late summer and fall are associated with an earlier molt. Seasonal and annual differences in catchability associated with molting need to be accounted for if trap catch rates are used as indices of lobster abundance and for estimation of exploitation rates.

ACKNOWLEDGMENTS

We thank the fishermen of Little River and Englishtown for their cooperation during this study. The Little River group was particularly helpful in making their vessels available to us free of charge to obtain samples outside the fishing season. R. Miller, D. Pezzack, and two anonymous reviewers are thanked for helpful comments on the manuscript.

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EFFECT OF NITRITE ON GROWTH AND OXYGEN CONSUMPTION FOR JUVENILE GREENLIP ABALONE, *HALIOTIS LAEVIGATA* DONOVAN

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ABSTRACT Juvenile greenlip abalone, *Haliotis laevigata* Donovan (mean whole weight, 5.61 g) were grown for 2–3 mo in bioassay tanks. Specific growth rate (SGR), measured on a whole-weight ($p < 0.05$) or shell length ($p < 0.01$) basis, was significantly affected by nitrite (NaNO_2). Modeling of the whole weight indicated relatively uniform growth depression (average SGR weight of 67.2% relative to the control, 0.024 mg of $\text{NO}_2\text{-N L}^{-1}$), regardless of concentration in the range of 0.56–7.80 mg of $\text{NO}_2\text{-N L}^{-1}$. This pattern of growth depression, which is independent of nitrite concentration once growth is reduced relative to controls, has been recorded by other researchers for penaeid shrimp and freshwater crayfish. SGR data for shell length exhibited a similar pattern, except that much more severe growth depression (average SGR length of 17.7% relative to the control) was recorded for the highest concentration (7.80 mg $\text{NO}_2\text{-N L}^{-1}$). Compared with several aquatic species studied by other authors, greenlip abalone are sensitive to nitrite on a growth basis. Oxygen consumption declined sharply with increasing nitrite concentration ($y = 82.452 \cdot e^{-0.154 \cdot x}$; range, 0.025–7.72 mg of $\text{NO}_2\text{-N L}^{-1}$). However, neither food consumption (as a percentage of initial biomass, corrected for mortality) nor survival was significantly affected by nitrite concentration ($p > 0.05$).

KEY WORDS: abalone, *Haliotis laevigata*, nitrite, growth molluscs, oxygen

INTRODUCTION

The major source of nitrogenous compounds in aquaculture systems is usually from the catabolism of protein contained within feed, with ammonia being the major end product (Colt and Armstrong 1981). In aerobic environments, nitrifying bacteria oxidize ammonia to nitrogen oxides, including nitrite (by *Nitrosomonas* spp.), and finally nitrate (by *Nitrobacter* spp.) (Brock and Madigan 1991). In flow-through systems, ammonia will be the principal toxic metabolite by-product, but in recirculating systems, both ammonia and nitrite may occur at toxic levels (Colt and Armstrong 1981). As the end product of nitrification, nitrate is the least toxic of inorganic nitrogen compounds to juvenile aquatic animals and will only be a problem in recirculating systems because of its effects on osmoregulation (Colt and Armstrong 1981). The conversion of nitrite to nitrate can be the rate-limiting step when conditioning biofilters, as a build-up of ammonia can inhibit this conversion and cause a subsequent build-up of nitrite (Anthonsen et al. 1976, de Guingand and Maguire 1992).

As a component of nitrogenous wastes, nitrite is known to affect oxygen transport (Jensen et al. 1987, Jensen 1995), cause tissue damage (Michael et al. 1987), and result in the oxidation of other compounds (Crawford and Allen 1977, Colt and Armstrong 1981). Nitrite penetrates cell membranes and is bioaccumulated in the extracellular spaces, particularly in gill, liver, brain, and muscle tissues of fish (Jensen 1995). The ionized form of nitrite, although not freely diffusible, is actively transported across gill membranes (Wedemeyer and Yasutake 1978, Bath and Eddy 1980, Jensen 1995, Schoore et al. 1995).

In fish, nitrite combines irreversibly with hemoglobin to cause methemoglobinemia because the hemoglobin is no longer able to combine with oxygen (Needham 1961). Hemocyanin is the respiratory pigment in some invertebrates including abalone, and although there is some suggestion of nitrite forming a complex with

hemocyanin thereby affecting oxygen consumption, others consider this effect to be negligible (Needham 1961), or less deleterious than the complexing of nitrite and hemoglobin (Jensen 1995). Hemocyanin can take up oxygen even in the presence of strong oxidizing agents (Needham 1961), and hence, oxygen transport by hemocyanin is generally much less affected by nitrite than is oxygen transport by hemoglobin. The formation of methemocyanin occurs primarily at low pH, in the presence of a large excess of nitrite, and appears unimportant at physiological pH (Jensen 1995). However, a severe excess of nitrite may occur in some aquatic animals, because nitrite accumulates in extracellular fluid in freshwater fish and crustaceans in concentrations well above ambient (3–33 times higher in 1–7 days) (Gutzmer and Tomasso 1985, Jensen et al. 1987, Harris and Coley 1991, Schoore et al. 1995, Jensen 1996, Stormer et al. 1996), although this has not yet been established for abalone. The competitive exclusion of nitrite ion uptake via the chloride cells by chloride ions increases the tolerance of marine fish to nitrite (Wedemeyer and Yasutake 1978) while also preventing extracellular nitrite levels of marine fish and crustaceans from greatly exceeding ambient concentrations (Eddy et al. 1983, Chen and Chen 1992a, Chen and Chen 1992b, Jensen 1996).

Although data on effects of nitrite on crustaceans and fish are readily available, information regarding molluscs, including abalone, is limited. Abalone culture is increasing in response to declining worldwide fishery production (Hone and Maguire 1996). Increasing production and subsequent reliance on protein-rich, formulated feeds and the introduction of recirculating culture systems increase the likelihood of abalone encountering elevated nitrite concentrations. In this study, we aimed to assess the chronic toxicity of nitrite to juvenile greenlip abalone, *Haliotis laevigata*, the most widely farmed abalone in Australia, in terms of growth and oxygen consumption.

TABLE 1.
Abbreviations used in the text.

Abbreviation	Definition
NO ₂ -N	Nitrite nitrogen
SGRW	SGR (WWBW% day ⁻¹) $\frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{days}} \times 100$
SGRL	SGR (shell length % day ⁻¹) $\frac{\ln(\text{final length}) - \ln(\text{initial length})}{\text{days}} \times 100$
SE	Standard error

All values given as mean \pm SE, unless otherwise stated.

MATERIALS AND METHODS

The juvenile greenlip abalone used in these experiments were approximately 3 y old, from a commercial hatchery at Bicheno, Tasmania, Australia, where the research was conducted (148°18'E, 41°53'S). The initial mean length and weight of the abalone were 35.0 ± 0.1 mm (mean \pm SE) and 5.61 ± 0.06 g (mean \pm SE) ($n = 719$). For 2–3 mo before experimentation, these abalone were maintained on a mixture of three formulated abalone feeds (ABCHOW, Deakin, Promak) and benthic diatoms. Abalone used for this experiment were relaxed with aerated warm water (23–25°C) until they could be easily removed from tank surfaces. Subsequently, they were tagged (Hallprint, Adelaide, Australia) and then randomly distributed to 18 bioassay units after being blotted and weighed to the nearest 0.01 g (whole wet body weight, WWBW) and measured with callipers to 0.1 mm for determining growth indices (Table 1). Most of the abalone were exposed to specific nitrite (NO₂⁻) concentrations for 82 days and then again weighed and measured to assess individual growth.

Bioassay System

Abalone were held in cages (100 mm diameter \times 35 cm polyvinyl chloride tubes, with 6-mm mesh floor and 8-mm mesh wall sections) suspended vertically within 70-L bioassay tanks (Harris et al. in press). Forty abalone were contained within a single cage in each tank. Oceanic seawater was filtered through a commercial sand filter and delivered to six 1,100-L reservoirs. The reservoirs were drained and refilled each day with seawater dosed with the

appropriate amounts of sodium nitrite (NaNO₂). Each reservoir was connected to a constant head chamber, which supplied three bioassay chambers via standard lengths of black 4-mm polypropylene tubing, which were replaced fortnightly to avoid nitrification by biofilms (Harris et al. in press). The bioassay tanks had conical ends to concentrate solid wastes and to avoid air spaces. Daily flow rates averaged 181.8 ± 1.5 mL min⁻¹ (mean \pm SE; $n = 54$), giving an effective replacement rate of 90% of bioassay tank volume in 12 h, in accordance with Sprague's (1969) 90% recommended replacement in 8–12 h. The experiment was conducted with 200- to 300-W aquarium heaters in the bioassay tanks and head adjustment columns, respectively, to maintain a constant temperature (Table 2). The average daily recovery of NO₂-N between reservoirs and bioassay tanks varied from 84.0 to 95.6% ($n = 5$).

Water Quality Analysis

The nitrite concentration of one replicate tank from each treatment, along with pH, temperature, and dissolved oxygen in all tanks were measured on each of 72 days. Water samples were collected in acid-washed glassware, and nitrite was measured by the diazotization method (Grasshoff 1989). A pH meter and combination glass electrode (Hanna Instruments HI 9025) were calibrated with phosphate (pH = 7.00) and borate (pH = 9.28) buffers daily before use (Bruno and Svoronos 1989). Ammonia was measured occasionally by the indophenol blue spectrophotometric method (Dal Pont et al. 1974). A WTW Microprocessor Oximeter OX1 96 oxygen electrode, used for daily measurements, was calibrated before use in "air-saturated" seawater and checked periodically using Winkler's titration.

Experiment 1: Chronic Nitrite Exposure

One control and five experimental treatments were established (Table 2); average nitrite concentrations ranged from 0.024 to 7.80 mg of NO₂-N L⁻¹. All cages were checked daily for mortality.

All tanks were fed a proprietary, formulated abalone diet (ABCHOW; 35% protein on a dry matter basis) every 2–3 days. The feeding ration was adjusted in response to food consumption data as the trial progressed. Food consumption was estimated on four occasions (Days 16, 38, 60, and 63) from uneaten food removed from the base of the cages after 2 days and dried for 24–48 h at 55–60°C. Residual food weight was not corrected for soluble

TABLE 2.
Water quality parameters within the chronic nitrite exposure trial (Experiment 1).

Treatment	NO ₂ -N mg L ⁻¹			pH	% Survival	Food Consumption (g g ⁻¹ day ⁻¹)
		Min	Max			
1	0.024 \pm 0.005	0	0.45	7.94	100 \pm 0	0.037 \pm 0.001
2	0.56 \pm 0.018	0.36	1.68	7.90	89.17 \pm 10.83	0.035 \pm 0.001
3	1.12 \pm 0.017	0.49	1.43	7.88	66.67 \pm 18.05	0.043 \pm 0.006
4	1.87 \pm 0.059	0.61	2.74	7.88	77.5 \pm 16.65	0.032 \pm 0.002
5	4.15 \pm 0.094	1.89	5.63	7.92	90.83 \pm 9.17	0.034 \pm 0.002
6	7.80 \pm 0.233	0	10.66	7.91	73.37 \pm 21.74	0.052 \pm 0.016

* Values are means \pm SE ($n = 3$) for each treatment.

† Average temperature and dissolved oxygen were 17.7 ± 0.1 °C (range, 17.0–19.1; $n = 69$) and 7.6 ± 0.03 mg L⁻¹ (range, 6.9–8.4; $n = 58$).

‡ Data were transformed before statistical analyses.

§ Based on measurements on four occasions, average ammonia concentration was similar (0.002 mg of FAN L⁻¹) in all treatments except the control (0.004 mg of FAN L⁻¹) (FAN, free ammonia-nitrogen, or unionized ammonia-nitrogen).

and particulate nutrient losses over the 2 days. Apparent food consumption (amount of food supplied minus residual food as grams dry weight) was divided by the initial tank biomass, less the initial weights of any mortalities to that point.

Tanks were cleaned, on average, every 6 days. Cleaning involved lowering the water level, siphoning enough water into a 20-L bucket to cover the cages, removing cages to the bucket, draining the tank, scrubbing the tanks and cages, refilling the tanks directly from the preheated adjustment columns, and returning the cages to the tanks, in less than 10 min for any tank. All tank valves were briefly opened each day to remove the organic build-up from the base of the tanks.

Experiment 2: Respirometry at End of Chronic Bioassay

The respirometer system included five elliptical perspex chambers (of 2.31 L) normally set up with two chambers for each treatment and one chamber as control (no animals) (Harris et al. in press). Flow entered each chamber near the base, was continuous, was controlled by a rotameter, and was measured manually twice daily. Flow exiting the top of the chamber was diverted by solenoids to either waste (50 min/h) or to a flow cell containing an Orion oxygen electrode for 10 min/h for data recording. The electrode was automatically calibrated with fully aerated seawater for 10 min in each hour. Values for tanks containing animals were corrected for the oxygen uptake of the control tank, and the final values were divided by the wet weight of animals to provide $\text{mg kg}^{-1} \text{h}^{-1}$. The data represented here are average values for the 24 h representing the third (and last) day of each trial.

Commencing Day 64, 29–30 abalone from Treatments 3 (208.64 g) and 2 (195.68 g), respectively, were transferred to respirometer chambers for 3 days (two duplicate chambers for each of two treatments plus one vacant control chamber in each 3-day cycle). Abalone that did not attach to transferable plastic strips were removed manually, either by sliding them directly from the substrate or by inserting a thin, plastic card underneath each abalone's foot. Daily measurements of nitrite concentration, pH, and temperature levels of effluent water from the reservoirs were undertaken, because the chambers were sealed units (Table 3). On Day 69, 30 abalone from two replicates of Treatments 6 (194.20 g) and 5 (233.96 g), respectively, were transferred to the respirometer for the next 3 days, and on Day 73, 30 abalone from two replicates of Treatments 1 (238.42 g) and 4 (197.64 g), respectively, were transferred to the respirometer for the next 3 days.

TABLE 3.

Water quality parameters in respirometer chambers (Experiment 2).

Treatment	$\text{NO}_2\text{-N}$ (mg L^{-1})*	Temperature ($^{\circ}\text{C}$)
1†	0.025 ± 0.0002	20.1
2‡	0.52 ± 0.017	20.7
3‡	1.01 ± 0.023	21.9
4†	1.99 ± 0.107	17.1
5§	4.29 ± 0.122	19.0
6§	7.72 ± 0.198	20.5

* Means \pm SE, $n = 3$.

† Flow = $169.1 \pm 3.6 \text{ mL min}^{-1}$.

‡ Flow = $164.1 \pm 1.7 \text{ mL min}^{-1}$.

§ Flow = $174.0 \pm 4.2 \text{ mL min}^{-1}$.

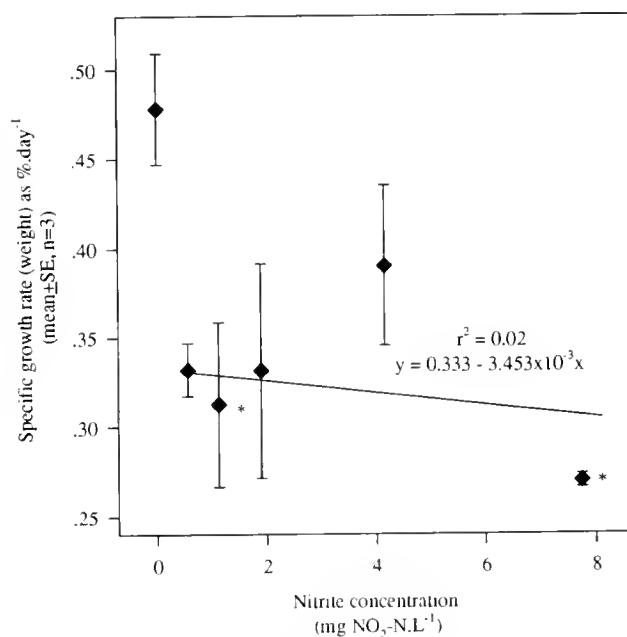
Statistical Analysis

Data were subjected to one-factor analysis of variance after meeting assumptions of normality using the Shapiro-Wilk test (Zar 1996) and homogeneity of variance using Cochran's test (Underwood 1981). Replicates were considered to be independent, and nitrite concentration was analyzed as a fixed factor. Survival data and WWBW:shell length ratio were transformed ($\sqrt{\arcsin}$ and log, respectively) before analysis. Results for each nitrite concentration were compared against data for the control ($0.024 \text{ mg NO}_2\text{-N L}^{-1}$) using Dunnett's test (Sokal and Rohlf 1995). Preliminary analysis indicated that initial size did not affect specific growth rate in this trial. All analyses, including assessment of initial size, survival, unionized ammonia-N, pH, dissolved oxygen, and temperature as covariates (Sokal and Rohlf 1995), were conducted with JMP 3.0 software (SAS Institute).

RESULTS

Experiment 1: Chronic Nitrite Exposure

Specific growth rate (SGR) was significantly affected by nitrite whether SGR was measured on a whole-weight ($p < 0.05$) or shell-length ($p < 0.01$) basis. Growth rates (weight) were, on average, 67.2% of controls ($0.024 \text{ mg of NO}_2\text{-N L}^{-1}$), regardless of nitrite concentration in the range $0.56\text{--}7.80 \text{ mg of NO}_2\text{-N L}^{-1}$, although there was considerable variation among replicates (Fig. 1). SGR data for shell length exhibited a similar pattern, with a plateau of growth rates from 61.4 to 54.8% of control values for Treatments 2–5 ($0.56\text{--}4.15 \text{ mg of NO}_2\text{-N L}^{-1}$), except that much more severe growth depression (17.7% of control growth rate) was recorded for the highest concentration ($7.80 \text{ mg of NO}_2\text{-N L}^{-1}$).



* = significantly different to control ($P < 0.05$)

Figure 1. SGR (weight) of juvenile greenlip abalone, *H. laevigata*, subjected to chronic nitrite exposure (mean \pm SE, $n = 3$). The regression line is plotted for $0.56\text{--}7.80 \text{ mg of NO}_2\text{-N L}^{-1}$.

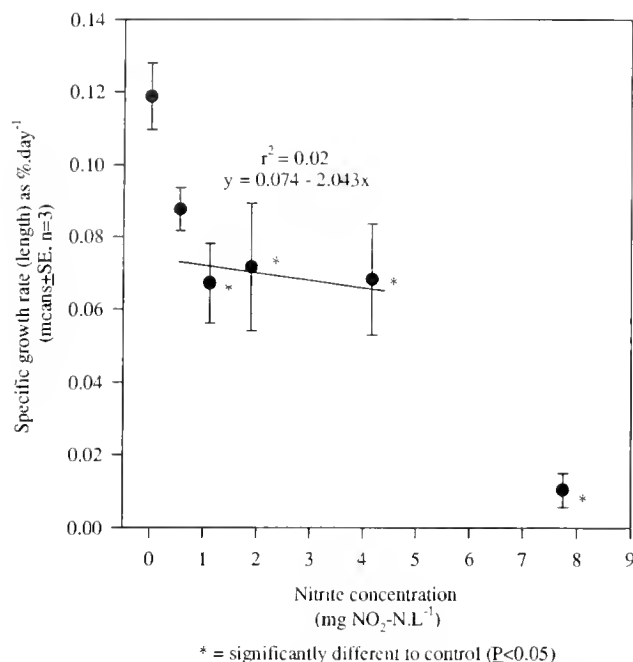


Figure 2. SGR (length) of juvenile greenlip abalone, *H. laevigata*, subjected to chronic nitrite exposure (mean \pm SE, $n = 3$). The regression line is plotted for 0.56–4.15 mg of $\text{NO}_2\text{-N L}^{-1}$ in order to define the plateau.

(Fig. 2). For both sets of data, linear models were fitted to these apparent plateaus.

WWBW:shell length data demonstrated significant differences from the control ($p < 0.01$) at Treatments 2, 3, and 6 (0.56, 1.12, and 7.80 mg of $\text{NO}_2\text{-N L}^{-1}$), although no significant differences ($p > 0.05$) were recorded for Treatments 4 and 5 (1.87–4.15 mg of $\text{NO}_2\text{-N L}^{-1}$) (Fig. 3). As with the growth data, there were large differences between the control and the remaining treatments, and hence, the quadratic model applied to the data does not include the control.

There was no significant effect of nitrite concentration on food consumption ($p > 0.05$), although it should be noted that the variance was much higher for Treatment 6 (7.80 mg L^{-1}). Survival was not significantly affected ($p > 0.05$) by nitrite concentration (mean \pm SE = $82.99 \pm 5.07\%$; $n = 6$), although the controls were the only treatment with 100% survival.

Experiment 2: Oxygen Consumption Rates at End of Chronic Bioassay

Oxygen consumption rate decreased with increasing nitrite concentration ($p < 0.01$) (Fig. 4). Significant reductions ($p < 0.05$) in oxygen consumption rate occurred in Treatments 5 and 6 (4.29–7.72 mg of $\text{NO}_2\text{-N L}^{-1}$) compared with the controls. An exponential decay model was used because of the similarity in oxygen consumption for the two highest concentrations (4.29–7.72 mg of $\text{NO}_2\text{-N L}^{-1}$). This model suggests that oxygen consumption will plateau at nitrite concentrations above 7.72 mg of $\text{NO}_2\text{-N L}^{-1}$, but without more data, this cannot be presumed.

DISCUSSION

Growth rates of control animals (SGRW = $0.48 \pm 0.035\%$ day $^{-1}$; SGRL = $0.122 \pm 0.011\%$ day $^{-1}$) were comparable with those found in a concurrent trial with greenlip abalone of a similar

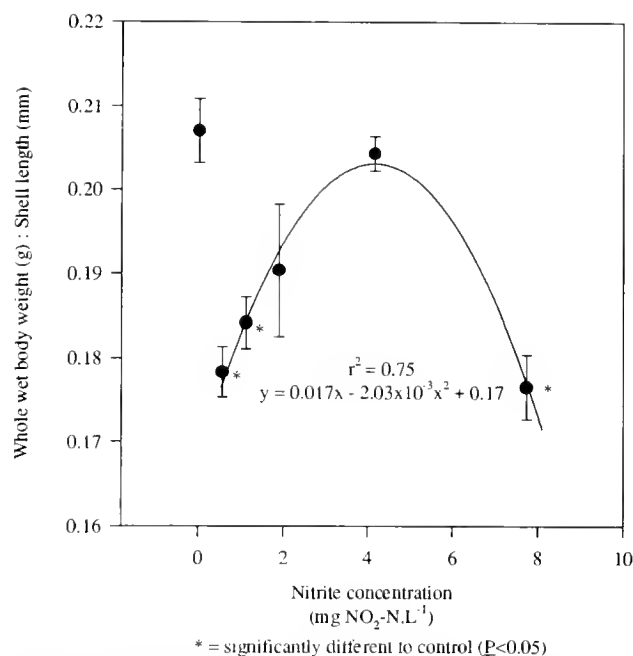


Figure 3. WWBW:shell length of juvenile greenlip abalone, *H. laevigata*, subjected to chronic nitrite exposure (mean \pm SE, $n = 3$).

size, conducted in outdoor ambient tanks (SGRW = $0.305 \pm 0.031\%$ day $^{-1}$; SGRL = $0.107 \pm 0.019\%$ day $^{-1}$) (Maguire et al. 1996). This suggests that the bioassay environment was not directly stressful for the control animals. However, faster growth rates for this species have been recorded in other culture systems at a higher constant temperature (Coote et al. 1996).

Nitrite has been shown to adversely affect growth or food consumption in several aquatic species; however, at least two quite

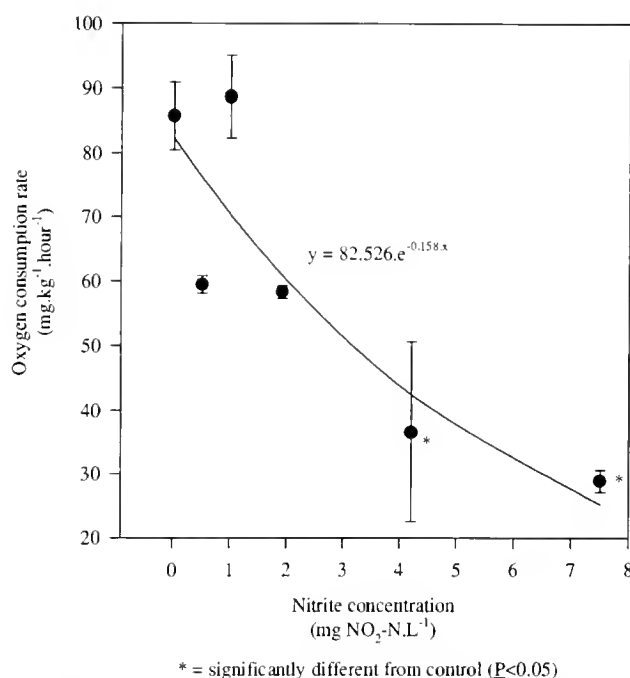


Figure 4. Oxygen consumption of juvenile greenlip abalone, *H. laevigata*, exposed to nitrite (mean \pm SE, $n = 2$).

different dose response patterns have been reported. Wickins (1976) reported a 50% reduction in growth of the marine shrimp *Penaeus indicus* Milne-Edwards at $6.4 \text{ mg of NO}_2\text{-N L}^{-1}$, and the growth data (Fig. 5) reflect this trend in whole-weight and shell-growth data for greenlip abalone. As nitrite concentration is increased, growth may be depressed; however, as nitrite concentration is increased further, growth inhibition is not necessarily exacerbated (Figs. 1 and 2). Chen and Chen (1992c) found significant growth reductions for *Penaeus monodon* Fabricius juveniles at and above $4 \text{ mg of NO}_2\text{-N L}^{-1}$, again with a plateau being evident in the dose response pattern, particularly for total length data. A similar pattern was obtained by Liu and Avault (1996) for the freshwater crayfish *Procambarus clarkii*. The respiratory pigment in abalone, penaeid shrimp, and freshwater crayfish is hemocyanin. The only study of fish that we can locate on the adverse effects of nitrite on growth is on channel catfish (Colt et al. 1981). In their study, a more typical dose response was obtained, with an initial plateau indicating negligible effect on growth at lower nitrite concentrations, followed by a linear decline in growth at high concentration (Fig. 6). The only available study on molluscs is on the acute toxicity and algal clearance rates of the bivalves, *Crassostrea virginica* Gmelin and *Mercenaria mercenaria* Linné, where levels of $280 \text{ mg of NO}_2\text{-N L}^{-1}$ caused clearance rate reductions of 89 and 54% for juveniles and 66 and 53% for adults, respectively (Epifanio and Srna 1975).

The apparent decline in oxygen consumption with increasing nitrite-nitrogen concentration may reflect compromised efficiency of the respiratory pigments. The Australian redclaw crayfish, *Cherax quadricarinatus*, demonstrated a similar decrease in oxygen consumption when exposed to nitrite at $100 \text{ mg of NO}_2\text{-N L}^{-1}$, although static conditions were used (Meade and Watts 1995). Other studies on carp, *Cyprinus carpio* L., found that when methemoglobin levels rose with exposure to nitrite, arterial oxygen content declined (Jensen et al. 1987, Williams et al. 1992). Similar patterns occur in penaeid shrimp when exposed to nitrite, as pH, oxyhemocyanin, protein, and oxyhemocyanin:protein levels within

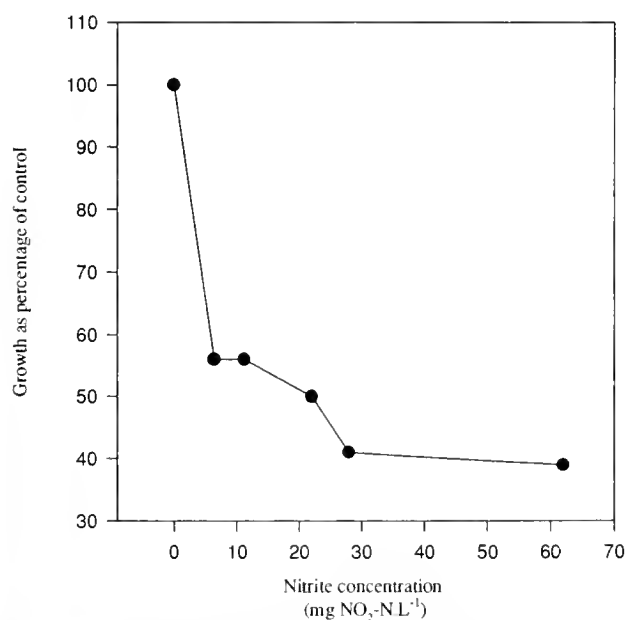


Figure 5. Growth of *Penaeus indicus* subjected to nitrite exposure (after Wickins 1976).

the hemolymph decline, with the probable result of methemocyanin formation (Chen and Cheng 1995). The hypothesis supplied by Fox (1954), suggesting that many hemoglobin-containing fishes, when swimming quietly, obtain enough oxygen for their needs in the blood plasma, and probably only require an additional supply when they are moving actively, may be useful in this case. In the wild, greenlip abalone exhibit limited movement (mean, 0.5 m mo^{-1}) that tends to increase with declining crevice abundance (Shepherd 1986, Shepherd and Godoy 1989). Greenlip abalone in this study had limited scope for movement in the bioassay cages, and any adverse effects on oxygen loading may have been ameliorated by restricted movement and hence oxygen demand.

In an equivalent study on the effects of ammonia on greenlip abalone (Harris et al. in press), growth results were consistent with food consumption and respiration rate data (as SGRW). As ammonia increased, food consumption was depressed and respiration rate increased, both of which would have contributed to the resultant depressed growth. In this study, nitrite depressed growth and respiration rate but did not affect food consumption. Neither of these trends would necessarily cause depressed growth. It is likely that inefficient use of available energy is occurring; this is consistent with the higher rate of protein catabolism, indicated by ammonia excretion, as observed in penaeid shrimp exposed to nitrite (Chen and Cheng 1995). Clearly, further research in this area is required for juvenile greenlip abalone.

The data for WWBW:shell length suggest that nitrite can affect whole-animal growth (weight) and shell growth (length) differently. We argued that ammonia affected shell growth more than whole-body growth (weight) at low ammonia concentrations, but that this pattern was reversed at high concentrations (Harris et al. in press). The pattern for nitrite is more complex; severe depression of shell growth at the highest concentration (Fig. 2) is not reflected in WWBW:shell length. The low ratio at this concentra-

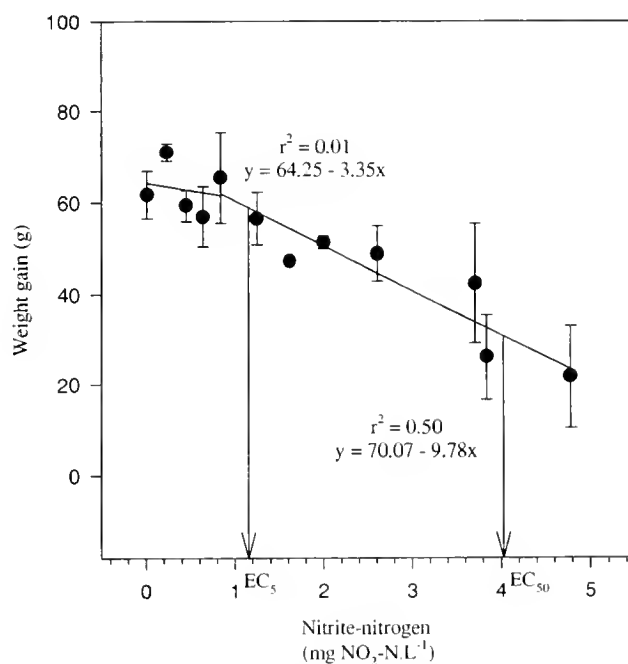


Figure 6. Growth of channel catfish, *Ictalurus punctatus*, subjected to nitrite exposure for 31 days (mean \pm SE; $n = 3$) (after Colt et al. 1981). EC_{50} and EC_{50} values were calculated from these data.

TABLE 4.
Growth of various juvenile aquatic animals subjected to nitrite exposure.

Species	Level of NO ₂ -N (mg L ⁻¹)	Effect	Author(s)
<i>Penaeus aztecus</i>	4.8	No growth reduction	Wickins 1976
<i>Penaeus merguensis</i>	4.8	No growth reduction	Wickins, 1976
<i>Penaeus monodon</i> *	22.45	EC ₅₀ (weight)	Chen and Chen 1992c
	26.2	EC ₅₀ (length)	
<i>Penaeus indicus</i>	>6.4	50% growth reduction (weight)	Wickins 1976
<i>Macrobrachium rosenbergii</i>	15.4	Predicted incipient (4 wk) LC ₅₀	Wickins 1976
<i>Procambarus clarkii</i>	>2.97	Significant growth reduction	Liu and Avault 1996
<i>Ictalurus punctatus</i>	1.17	Estimated EC ₅ (weight)	Colt et al. 1981
	4.01	Estimated EC ₅₀ (weight)	
<i>Haliotis laevis</i>	0.56	Growth depression (length and weight)	This study
	1.12	Significant decline in wet weight	

* EC₅₀ value was outside of experimental range.

tion may reflect a limitation on whole-body growth imposed by depressed shell growth rates in gastropods (Palmer 1981, Preston et al. 1996). Liu and Avault (1996) reported changes in length gain/weight gain ratio for *Procambarus clarkii* exposed to nitrite.

The results for *H. laevis* suggest that this species is more sensitive to nitrite than are several other aquatic animal species (Table 4). Similarly, this species is quite sensitive to ammonia (Harris et al. in press), and hence in commercial growout systems, it will be important to minimize nitrogenous wastes. This can be achieved by reduction of dietary protein content (Jirsa et al. 1997), minimization of accumulation of organic matter (through appro-

priate feed rates, better digestibility, or efficient cleaning systems), or efficient biofiltration in recirculating systems.

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WITHERING SYNDROME OF THE BLACK ABALONE, *HALIOTIS CRACHERODII* (LEACH): WATER TEMPERATURE, FOOD AVAILABILITY, AND PARASITES AS POSSIBLE CAUSES

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ABSTRACT Withering syndrome (WS) has affected black abalone since the mid-1980s. We investigated the potential roles of elevated water temperature, food availability, and parasites (renal coccidia and rickettsiales-like procaryotes or RLPs) in this disease. Results from a temperature-feeding experiment suggested that elevated water temperature was not a direct cause of WS, but accelerated mortality. At a particular water temperature, both fed and starved abalone had similar survival. Abalone with WS fed on kelp until the animal reached the terminal stages of the disease, when visible atrophy of the foot muscle was easily observed. However, fed abalone held at an elevated water temperature, 20°C, had decreased survival relative to those held at 13°C. The lack of food in our investigations did not appear to be a direct cause of WS. In addition, no consistent statistically significant associations were identified between abalone condition and intensity of coccidian infection in both field and laboratory studies. No association was found between condition of the digestive gland and intensity of the RLP infection in our laboratory study. However, all abalone with degenerated digestive glands had visible signs of WS. Time to abalone death did not correlate with intensity of RLP infection, except in a pool of the 13°C treatments and possibly the 13°C starved treatment. Thus, at lower seawater temperatures, the RLP may affect survival. The significance of this observation may have resulted from small sample sizes. Only 22 abalone were included in the 13°C treatment, and 4 of these were in the starved treatment. These data suggest that further investigation of the role of the RLP in WS is needed.

KEY WORDS: withering syndrome, abalone, coccidia, rickettsia, starvation

INTRODUCTION

Declines in populations of black abalone, *Haliotis cracherodii*, on several California Channel Islands were noted in 1986 by Haaker et al. (1992). In 1988, dramatic declines in the population density of black abalone were also reported in Diablo Cove, mainland California (Steinbeck et al. 1992). This black abalone mortality has spread to seven of the eight Channel Islands examined (VanBlaricom et al. 1993). Mortality has approached 99% relative to 1985 levels at several locations on the Channel Islands (Richards and Davis 1993, Altstatt et al. 1996) and 85% in Diablo Cove (Sommerville 1991). Clinical signs of moribund abalone at all locations included an atrophied and flaccid foot muscle, lack of gonad development, weakness, and decreased tactile responses (Haaker et al. 1992). Collectively, these symptoms have been termed "withering syndrome" (WS) (Haaker et al. 1992).

Because of the appearance of WS after the 1983 El Niño, several hypotheses regarding the possible causes of this disease were formed. These included elevated water temperature, starvation, and disease (Haaker et al. 1992, Lafferty and Kuris 1993). Abnormally high water temperatures and severe winter storms during the El Niño resulted in a loss of kelp canopies, the main

source of food for many abalone (Dayton et al. 1992). Thus, starvation and thermal stress were initially suspected as causative agents of WS (Tissot et al. 1991).

An infectious agent was also thought to be a cause of WS because of its spread from a central location to surrounding areas (Lafferty and Kuris 1993, Altstatt et al. 1996). Microscopic examination of fixed tissues from affected abalone supported this hypothesis. Atrophy of the pedal muscle and degeneration of the digestive gland (Gardner et al. 1995, VanBlaricom et al. 1993) were observed. Two previously undescribed microorganisms were also found: a new species of coccidian (*Pseudoklossia haliotis*; Friedman et al. 1995) within the kidneys (nephridia) (Friedman 1991, Friedman et al. 1993, Haaker et al. 1992, Steinbeck et al. 1992) and an unnamed rickettsiales-like procaryote (RLP) within digestive epithelia (VanBlaricom et al. 1993, Gardner et al. 1995). No other unusual parasites or pathological changes within tissues were observed (Steinbeck et al. 1992, VanBlaricom et al. 1993). Although recently shown to be nonpathogenic for red abalone, *Haliotis rufescens*, and pinto abalone, *Haliotis kamschatkana* (Friedman et al. 1993, 1995), the coccidian parasite, *P. haliotis*, was suggested as a possible cause of WS in black abalone (Steinbeck et al. 1992). A second pathogen, an RLP (an intracellular

bacterium), was proposed as a second possible causative agent of this disease in black abalone (Gardner et al. 1995). Physiological data of Kismohandaka et al. (1993) documented decreased feeding and increased ammonia excretion in conjunction with atrophy or catabolism of the pedal muscle in abalone with WS relative to those without WS. It is likely that the RLP that infects digestive epithelia of the abalone is associated with the physiological alterations observed by Kismohandaka et al. (1993).

This study describes investigations of three potential causes individually and in conjunction with one another: (1) water temperature, (2) food availability, and (3) parasites (renal coccidia and RLPs). We determined the geographic and host distribution of the coccidian, *P. haliotis*, in abalone, with a focus on black abalone. In abalone collected from field locations, associations between the intensity of coccidian infection and the condition of the abalone were assessed. In laboratory studies, we examined the relationship between food availability, water temperature, black abalone survival, and intensity of infections by both coccidia and RLPs.

MATERIALS AND METHODS

Histology

The foot muscle, gills, left and right kidneys, and digestive gland were excised, fixed in Davidson's solution (Shaw and Battle 1957), and processed for routine paraffin histology (Luna 1968). Deparaffinized 5- μ m sections were stained with Harris's hematoxylin and eosin (Luna 1968). Scaled counts of both coccidia (in the nephridia) and RLPs (in digestive epithelia) were enumerated from stained tissue sections as follows: The infection intensity for the coccidian at 200 \times magnification was scaled as: (1+), no detectable parasites; (2+), 1–10 parasites per field; (3+), 11–100 parasites per field; (4+), 101–1,000 parasites per field, or up to approximately 75% of the cells infected; and (5+), >1,000 cells per field, or nearly every cell was infected. The infection intensity of RLPs at 200 \times magnification was measured as the number of bacterial foci within host digestive epithelial cells and was scaled using the same intervals as for the coccidian. The condition of the digestive gland was examined and rated from (1+) to (3+). A digestive gland was characterized as normal (3+) if the organ was composed of numerous acini that contained both duct (α) and crypt (β) cells, ducts composed of ciliated, columnar epithelial cells that transport secretions to the cecum, and sparse amounts of connective tissue between tubules as described by Bevelander (1988). Moderate degeneration was characterized as (2+), and severe degeneration or loss of most normal tissue was scored as (1+).

Geographic Distribution: Animals

Abalone, *Haliotis* spp., were haphazardly collected at 28 sites from Baja California Sur north to British Columbia, Canada, between September 1988 and March 1991 (Fig. 1). The maximum length (L) in centimeters, total weight (TW), and shell weight (SW) in grams of each abalone were measured. The tissues were processed for routine paraffin histology and were scored for parasites as previously described in the histology section.

The "condition" of the abalone was defined by a visceral condition index: a ratio of the visceral weight to total weight (V/TW), in which V represents the weight of the foot muscle and visceral mass ($V = TW - SW$). The condition index was grouped

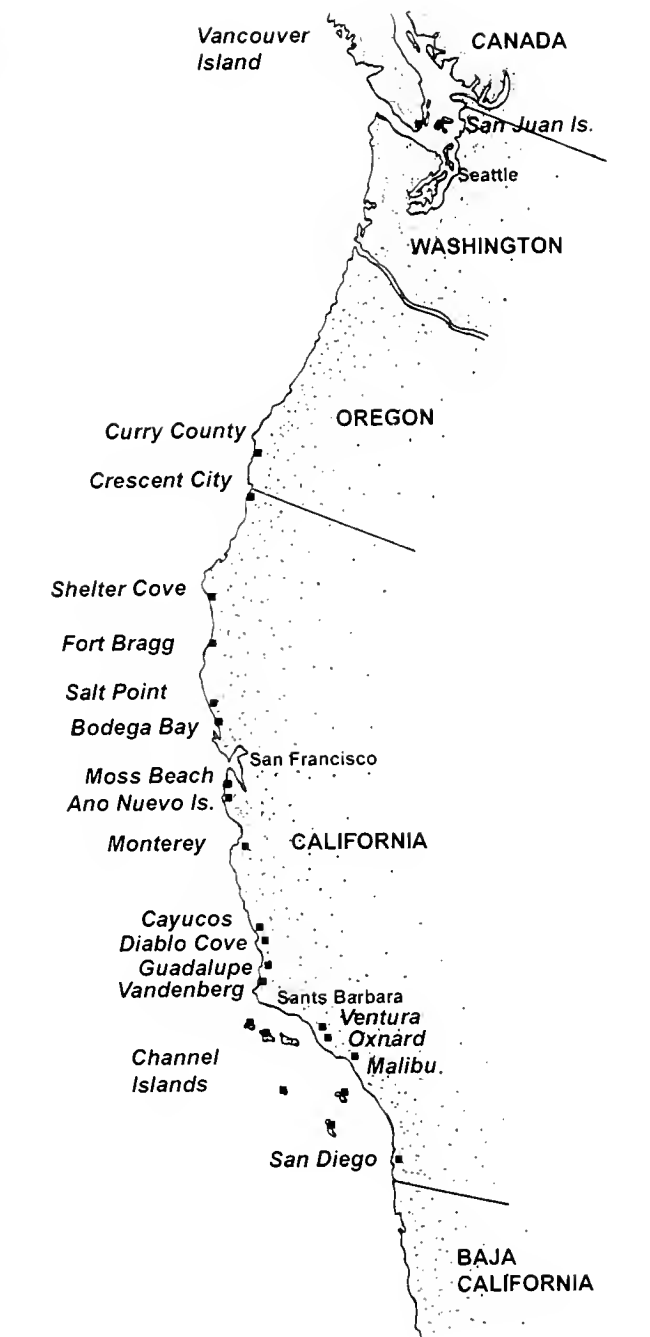


Figure 1. Map of abalone collection locations. Black squares indicate locations sampled as described in Table 1.

into three levels: (1+), healthy, if the ratio was 0.60 or higher; (2+), slightly shrunk, if the ratio was between 0.55 and 0.59; and (3+), very shrunk, if the ratio was below 0.55.

Although six species of abalone were sampled, only the 307 black abalone sampled from four Channel Islands and Malibu (mainland California) were subjected to formal statistical analyses. The islands studied were Santa Rosa Island, where WS was prevalent; San Clemente Island, where WS was recently observed; and Santa Catalina and San Nicolas Islands, before the first appearance of WS. Abalone were collected at one or more sites on each island, and some sites were visited more than once (Table 1).

TABLE 1.

Geographic and host distribution of a renal coccidian parasite of six species of abalone from the West Coast of North America from San Diego, CA north to British Columbia, Canada.

Location	Date	Species	N	L	R	L + R
California						
San Diego	5-22-89	<i>H. corrugata</i>	11	1	0	0
		<i>H. fulgens</i>	1	0	0	0
		<i>H. rufescens</i>	13	5	11	5
San Clemente*	1-24-90	<i>H. cracherodii</i>	48	28	12	10
Santa Catalina*	6-1-89	<i>H. cracherodii</i>	5	1	0	0
	8-19-89	<i>H. cracherodii</i>	26	21	0	0
	6-1-89	<i>H. corrugata</i>	4	0	0	0
		<i>H. fulgens</i>	5	0	0	0
Malibu	1-8-90	<i>H. cracherodii</i>	7	5/6†	2/5	2/7
San Nicolas*	4-4-90	<i>H. cracherodii</i>	36	26	23	19
Santa Rosa*	Fossil Reef	3-4-89	<i>H. cracherodii</i>	12	12/12‡	ND§
		5-11-89	<i>H. cracherodii</i>	39	19	39
		7-20-89	<i>H. cracherodii</i>	51	32	47
		10-18-89	<i>H. cracherodii</i>	14	10	12
		12-10-89	<i>H. cracherodii</i>	12	8	5
NW Talcott	7-19-89	<i>H. cracherodii</i>	30	22	17	11
	12-9-89	<i>H. cracherodii</i>	10	7	5	3
Johnson Lee	7-20-89	<i>H. cracherodii</i>	16	8	11	4
	12-12-89	<i>H. cracherodii</i>	1	1	0	0
Ventura	3-13-89	<i>H. rufescens</i>	61	37	37	37
Oxnard	10-17-88	<i>H. corrugata</i>	30	30	8	7
	10-17-88	<i>H. fulgens</i>	30	25	5	ND§
	10-17-88	<i>H. rufescens</i>	30	13/19	1	1
Vandenberg	6-22-89	<i>H. cracherodii</i>	20	12	4	ND§
Guadalupe	11-7-90	<i>H. rufescens</i>	60	60	60	60
Diablo Cove	9-12-88	<i>H. cracherodii</i>	15		7/15	ND§
	9-30-88	<i>H. cracherodii</i>	16		12/16	ND§
North Diablo Cove	10-13-88	<i>H. cracherodii</i>	6	3/4	4/6	1
Dead Cow Creek	10-13-88	<i>H. cracherodii</i>	10	7/7	10	7
Cayucos	12-7-89	<i>H. rufescens</i>	60	14	9	2
Monterey	6-7-89	<i>H. rufescens</i>	15	9	15	9
Ano Nuevo	11-2-88	<i>H. cracherodii</i>	10	7/7	10	7
Moss Beach	6-28-89	<i>H. rufescens</i>	16	14	0	0
Bodega Bay	6-7-89	<i>H. rufescens</i>	12	8	9	7
Salt Point	8-5-89	<i>H. rufescens</i>	8	3	0	0
Fort Bragg	6-7-89	<i>H. rufescens</i>	16	11	9	7
Fort Bragg	11-29-89	<i>H. rufescens</i>	13	1	0	0
	11-29-89	<i>H. walallensis</i>	1	0	0	0
	4-9-90	<i>H. walallensis</i>	3	3	3	3
Shelter Cove	7-26-89	<i>H. rufescens</i>	16	0	0	0
	4-9-90	<i>H. rufescens</i>	52	0	2	0
Crescent City	6-27-90	<i>H. rufescens</i>	3	0	0	0
	7-18-89	<i>H. rufescens</i>	2	0	0	0
	8-16-89	<i>H. rufescens</i>	7	0	0	0
	11-3-89	<i>H. rufescens</i>	1	0	0	0
Oregon						
Curry County	8-12-90	<i>H. rufescens</i>	8	0	0	0
	8-11-90	<i>H. walallensis</i>	1	0	0	0
Washington						
San Juan Island		<i>H. kamschatkana</i>	9	0	0	0
British Columbia, Canada						
Vancouver Island						
Bamfield	6-27-89	<i>H. kamschatkana</i>	8	0	0	0
Victoria		<i>H. kamschatkana</i>	60	0	0	0

Abbreviations: N, number of animals sampled. L, number of animals with coccidia in the left kidney. R, number of animals with coccidia in the right kidney. L + R, number of animals with coccidia in both kidneys.

* Indicates that this site is a Channel island.

† Indicates the number of kidneys with coccidians per number of kidneys observed on slides.

‡ Not determined.

§ Left or right kidney infections were not designated.

Statistical Analyses

The black abalone were analyzed for parasites and condition in different groupings of data for individual and pooled dates and sites. Separate analyses were done for each unique survey date and site. The dates of sites with multiple surveys were pooled for another analysis. Islands with multiple sites were also combined for an island analysis. Finally, an overall analysis used all of the black abalone data together.

The black abalone were analyzed for possible association between coccidian infection intensity and abalone condition. In each analysis, abalone condition was compared for coccidian infection intensity in both the left and the right kidneys individually and together. In the latter case, the highest intensity of infection of the two kidneys was used for the individual animal.

The nonparametric Mann-Whitney test and Fisher's exact test were used for all analyses except the pool of all data (SAS 1990). Healthy (condition level = 1) and shrunken (condition level = 2 or 3) abalone were compared for their levels of coccidian infection with the Mann-Whitney test. Because of small sample sizes for many data groupings, Fisher's exact test was used to test the independence of abalone condition and coccidian infection intensity. For Fisher's exact test, the abalone condition was grouped into healthy or shrunken, and the coccidian infection intensity was grouped into healthy or shrunken, and the coccidian infection intensity was grouped into uninfected (coccidian level = 1) or infected (coccidian level > 1).

The nonparametric Kruskal-Wallis test and the χ^2 test of independence for a contingency table were used to examine the pooled black abalone data. The Kruskal-Wallis test compared the three visceral condition levels of the abalone for intensity of coccidian infection. The coccidian infection intensity was examined for independence to the visceral condition of abalone with contingency table analysis.

Temperature-Food Availability Experiment

Black abalone in varying stages of health were collected 300–500 m southwest of Fossil Reef, Santa Rosa Island, on October 17, 1989, and transported to the Bodega Marine Laboratory, where they were maintained in 88-L aquaria. All aquaria received flow-through seawater (32 ppt) that had passed through sand filters. Abalone ($n = 195$) were weighed (TW), measured (L), tagged, and randomly divided into eight aquaria. Each aquarium contained 23–25 abalone. Subsequently, animals were acclimated to either 13 or 20°C over a 2-wk period and maintained at the target temperature for 1 wk before the initiation of the study.

Four aquaria were held at 13°C, and four were held at 20°C. Abalone in two aquaria within each temperature treatment were fed *ad libitum* a combination of primarily *Macrocystis pyrifera* and to a lesser extent, *Nereocystis luetkeana* and *Eggregia menziesii*. Abalone in the remaining aquaria were starved.

Abalone that died during the study were weighed (TW and SW), and their length was measured (L). Selected tissues were excised and processed for routine paraffin histology. As previously described, the condition of the digestive gland was assessed and the infection intensities of both renal coccidia and RLPs were measured and scaled.

The survivorship of black abalone was analyzed with the Kaplan-Meier estimate of the survivor function (Kaplan and Meier 1958). Four treatment groups were defined for the survival: 13°C fed, 13°C starved, 20°C fed, and 20°C starved. Specific combina-

tions of treatment groups were compared with the log-rank and the Wilcoxon tests (Kalbfleisch and Prentice 1980, SAS 1990). Possible effects of various covariates on survival were assessed. The Weibull regression model was used to model covariates that possibly influenced survival over time. Covariates in the Weibull model were tested with large-sample likelihood methodology (Kalbfleisch and Prentice 1980, SAS 1990). The effect of total coccidian infection on survival was also analyzed at each temperature separately, and for the combined data with both temperatures, with the log-rank and Wilcoxon tests.

We also tested for possible associations between abalone condition and both water temperature and coccidian infection intensity. Using the Mann-Whitney test, we compared the abalone held at the two water temperatures, 13 and 20°C, for initial abalone condition, final abalone condition, and change in condition. Using the Kruskal-Wallis test, we compared the five levels of coccidian intensity in each kidney separately and together for initial abalone condition, final abalone condition, and change in condition. In addition, Spearman rank correlation coefficients were calculated and tested for no relationship between the coccidian levels (in the left kidney, right kidney, both kidneys) and abalone condition (initial, final, and change in condition).

A subset of the experiment consisted of only abalone that were evaluated for both intensity of RLPs and condition of the digestive gland. The Spearman rank correlation assessed whether a (linear) relationship existed between RLP infection intensity and condition of the digestive gland. A zero rank correlation between RLP infection intensity and condition of the digestive gland was tested for the four treatment groups individually and for a pool of the treatment groups. The condition of the digestive gland was compared between fed and starved abalone with the Mann-Whitney test. This test was also used to compare the intensity of RLP infections in the fed and starved groups. Possible association between time to abalone death and intensity of RLP infection was examined with the Spearman rank correlation test.

The program SAS was used for all of the statistical analyses (SAS 1990). The statistical level of significance selected for the study was the 0.05 level.

RESULTS

Geographic Distribution: Renal Coccidia

The renal coccidia observed in all species of abalone examined in this study (Table 1) were morphologically indistinguishable, as previously described (Friedman et al. 1993). The prevalence of renal coccidiosis in California abalone was 69% (621/900), using all abalone sampled. The prevalence was relatively high, 77.5% (605/781), from San Diego north to Bodega Bay and was very similar between seasons (see Table 1, Fossil Reef, Santa Rosa Island). In contrast, between Bodega Bay and Crescent City, CA, coccidia were much less prevalent at 18% (21/119). In abalone from the southern California high and central California, the infection prevalences were similar at 78.5% (386/492) and 68.3% (164/240), respectively. No coccidia were observed north of California (Table 1). The geographic distribution of the coccidian (San Diego in southern California to Shelter Cove, Mendocino County, in northern California) far exceeded that of WS (southern California and Diablo Cove in southern central California; Fig. 1).

Except for two samples, no significant differences were found between healthy and shrunken black abalone for intensity of coccidian infection ($p > 0.05$ for all Mann-Whitney and Fisher's exact

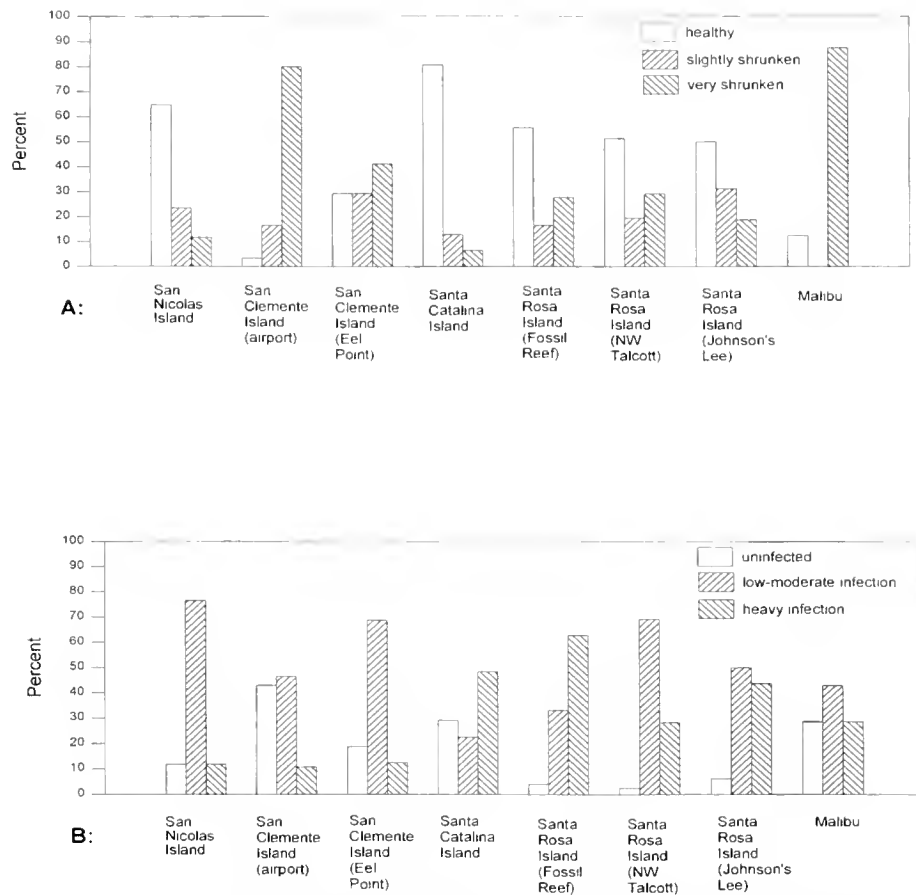


Figure 2. Percentages of abalone by location for abalone condition (A) and coccidian infection in both kidneys (B). The abalone condition levels (healthy, slightly shrunk, and very shrunk) correspond to levels 1+ to 3+, respectively. The coccidian infection levels (uninfected, low-moderate, and heavy) correspond to levels 1+, 2+ to 3+, and 4+ to 5+, respectively.

tests; Fig. 2). These two exceptions revealed conflicting results: The right kidneys of shrunk abalone had higher infection levels than those of healthy abalone collected from Fossil Reef, Santa Rosa Island, on May 11, 1989 ($p = 0.03$, Mann-Whitney test). In contrast, healthy abalone collected from San Nicolas Island on April 4, 1990, had a higher proportion of right kidneys infected than those of shrunk abalone ($p = 0.03$, Fisher's exact test). The grouped data, for dates grouped by sites and for sites grouped by island, showed no significant differences between healthy and shrunk abalone ($p > 0.05$ for all Mann-Whitney and Fisher's exact tests).

The three condition levels (healthy, slightly shrunk, or very shrunk) in the pooled data did not significantly differ in their coccidian infection intensity of the left kidney, the right kidney, and both kidneys together ($p = 0.88$, $p = 0.20$, and $p = 0.07$, respectively, Kruskal-Wallis test; Fig. 3). In addition, our data did not suggest a differential effect of the coccidian infection in one kidney relative to the other (e.g., right vs. left). High percentages (over 40%) of both the slightly shrunk and the very shrunk abalone had uninfected right kidneys (Fig. 3). In contrast, the percentages of abalone with uninfected left kidneys never exceeded 30% for each of the abalone condition levels. A higher percentage of the healthy abalone had very heavy coccidian infections in the right kidney compared with those of the very shrunk abalone. In addition, abalone condition was independent of coc-

cidian infection intensity in the left kidney alone, the right kidney alone, or both kidneys together ($p = 0.90$, $p = 0.16$, and $p = 0.06$, respectively, χ^2 test of independence).

Temperature-Food Availability Experiment

All animals that died in this study had signs of WS, including weight loss, visible atrophy of the foot muscle, weakness, and a decrease in condition index. Fed and starved abalone had similar survival at both 13 and 20°C ($p > 0.10$ and $p > 0.15$, respectively, log-rank and Wilcoxon tests). Results differed when fed and starved abalone were tested separately for a temperature effect. Temperature did not affect survival for starved abalone alone ($p > 0.10$, log-rank and Wilcoxon tests). In contrast, temperature affected survival for fed abalone ($p = 0.0001$, log-rank and Wilcoxon tests). Survival was better for fed abalone held at the lower temperature, 13°C, than for those held at 20°C. At 13°C, median survival time for fed abalone was 11.1 wk, but it was only 3.9 wk for those at 20°C (Fig. 4).

Weibull regression analysis showed that the three most important covariates for survival were water temperature, change in abalone condition, and intensity of total coccidian infection. When all three covariates were used in the model, only two, water temperature and change in abalone condition, were significant ($p = 0.002$ and $p = 0.003$, respectively); total coccidian infection had

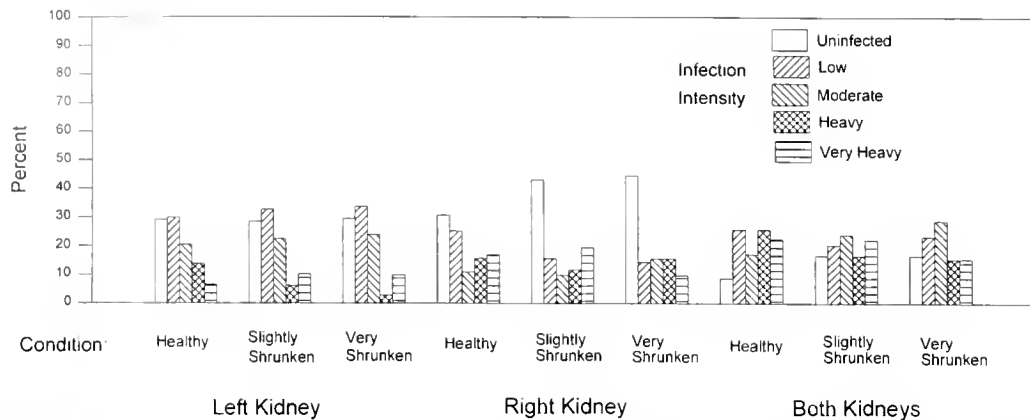


Figure 3. Percentages of abalone grouped by kidney site and abalone condition for renal coccidian infection intensity. The abalone condition levels (healthy, slightly shrunk, and very shrunk) correspond to levels 1+ to 3+, respectively. The coccidian infection levels (uninfected, low, moderate, heavy, and very heavy) correspond to levels 1+ to 5+, respectively.

no effect in this three-covariate model ($p = 0.26$). However, total coccidian infection did affect survival, as did change in condition, when they were the only two covariates in the model ($p = 0.04$ for both covariates). To further examine the role of renal coccidia in WS, we analyzed the effect of total coccidian infection by itself on survival. Total coccidian infection did not affect survival when water temperatures were considered separately ($p > 0.69$ for 13°C and $p > 0.71$ for 20°C, log-rank and Wilcoxon tests) or in combination ($p > 0.18$, log-rank and Wilcoxon tests).

Although animals were randomly distributed between aquaria, initial abalone condition differed between water temperatures, but the differences were small (sample means for 13 and 20°C were 0.60 and 0.65, respectively; $p = 0.002$, Mann-Whitney test). No significant difference was found for final abalone condition (sample means for 13 and 20°C were 0.54 and 0.52, respectively; $p = 0.25$, Mann-Whitney test). However, the change in abalone condition was significant between temperatures (sample means for 13 and 20°C were 0.06 and 0.10, respectively; $p = 0.0004$, Mann-Whitney test). On average, abalone held at the higher water temperature worsened more than abalone held at the lower temperature.

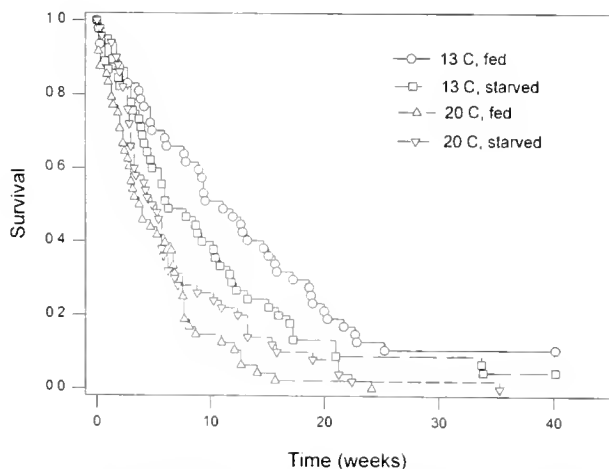


Figure 4. Kaplan-Meier survivorship curves from the Temperature-Food Availability Experiment. Each curve represents a pool of two replicate aquaria.

We did not find any significant differences in abalone condition by levels of coccidian infection intensity: initial abalone condition ($p = 0.53$, $p = 0.58$, and $p = 0.28$, for left kidney, right kidney, and both kidneys, respectively; Kruskal-Wallis test), final abalone condition ($p = 0.79$, $p = 0.30$, and $p = 0.16$, for left kidney, right kidney, and both kidneys, respectively; Kruskal-Wallis test), and change in abalone condition ($p = 0.99$, $p = 0.25$, and $p = 0.44$, for left kidney, right kidney, and both kidneys, respectively; Kruskal-Wallis test). Spearman rank correlations between coccidian infection levels in the left kidney, right kidney, and both kidneys and initial abalone condition, final abalone condition, and change in condition were low, with all rank correlations between -0.16 and 0.05 . All of the tests for no relationship were nonsignificant ($p = 0.07$ for correlation between coccidian intensity in the right kidney and change in abalone condition, and $p > 0.25$ for the rest; Spearman rank correlation test).

A second analysis considered only a subset of the black abalone that died during the study in which both the RLP and the digestive gland were evaluated. This resulted in a sample of 80 black abalone, of which 40 were starved and 40 were fed. Healthy abalone contained normal digestive glands (3+), as described previously (Bevelander 1988; Fig. 5A). Digestive glands with moderate degeneration (2+) were characterized by limited atrophy and necrosis of digestive acini, and, in some cases, inflammation (Fig. 5B). Digestive glands in which many or most digestive tubules were necrotic or lost, leaving only the connective tissue sheath, were scored as severely degenerated (1+; Fig. 5c).

Intensity of infection with RLPs (Fig. 6) was not (linearly) related to condition of digestive gland for both fed and starved abalone at 13°C ($p = 0.22$ and $p = 0.20$, respectively; Spearman rank correlations). Similar results were found at 20°C for fed and starved abalone ($p = 0.42$ and $p = 0.41$, respectively; Spearman rank correlations) or when all of the data were combined ($p = 0.14$; Spearman rank correlation). In black abalone with advanced signs of WS [very atrophied foot muscle (Fig. 7) and degenerated digestive gland (1+)], infections with the RLP ranged from no (0+) or light (1+) to severe (3+). The intensity of infection of RLPs was not significantly different between fed and starved abalone ($p = 0.35$; Mann-Whitney test). Also, fed abalone did not significantly differ from starved abalone in condition of the digestive gland ($p = 0.23$; Mann-Whitney test). Thus, starvation had no significant

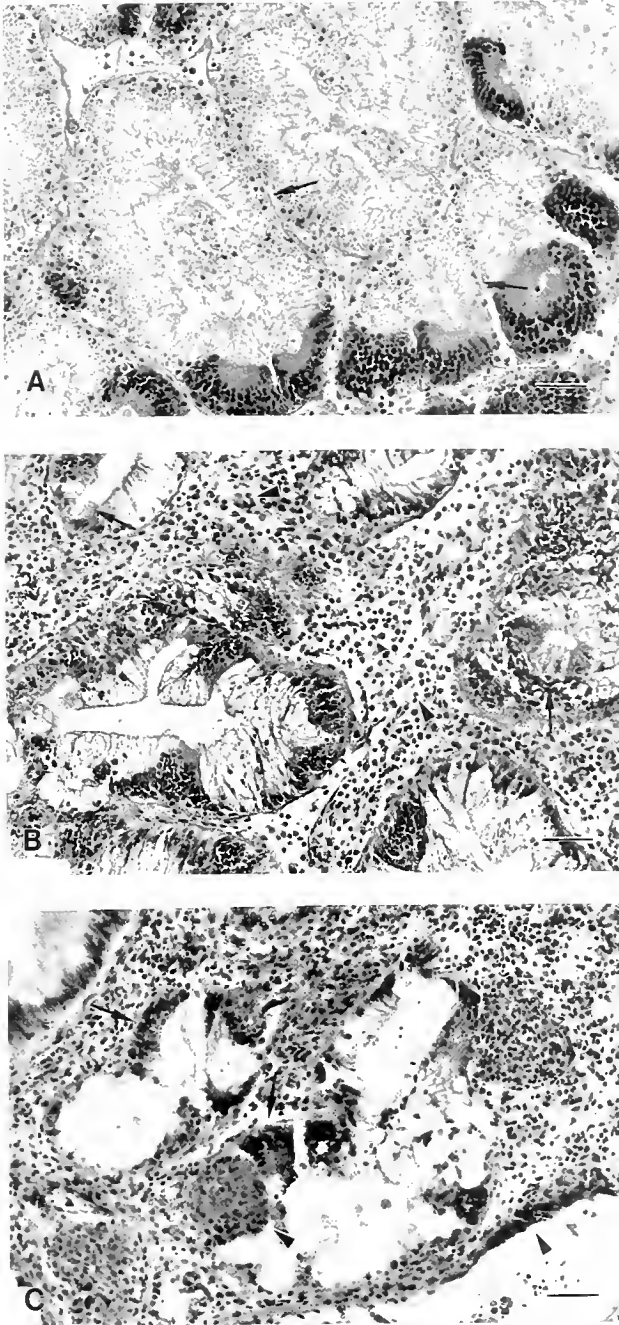


Figure 5. Photomicrograph of digestive glands from black abalone. (A) Healthy abalone. Note the lack of space between digestive tubules (arrow) and lack of tubule degeneration. (B) Moderate degeneration of digestive gland. Note atrophy of digestive tubules (arrow) and infiltration of hemocytes between tubules (arrowhead). (C) Advanced degeneration of digestive gland as evidenced by necrosis (arrowhead) and loss of secretory tubules (arrow). Hematoxylin & eosin. Bars = 50 µm.

influence on either infection intensity of the rickettsiales-like bacterium or condition of the digestive gland.

In addition, intensity of RLP infection did not correlate with time to abalone death in three of the four treatment groups and in a pool of all data ($p = 0.07$, $p = 0.74$, $p = 0.32$, and $p = 0.15$; Spearman rank correlations). A borderline association between RLP infection and time to abalone death was found for the 13 C

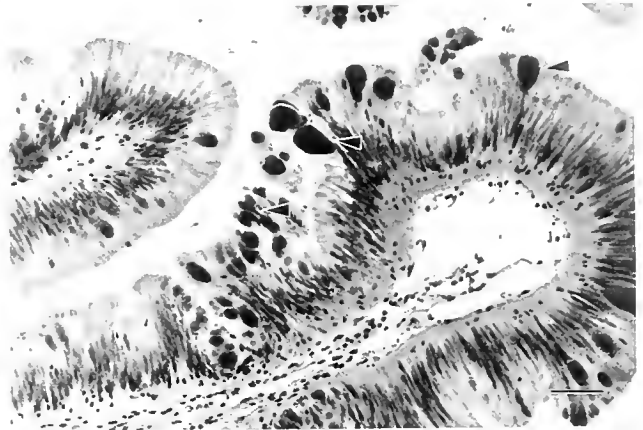


Figure 6. Photomicrograph of postesophagus of a black abalone with a heavy rickettsial infection (arrowheads). Hematoxylin & eosin. Bar = 50 µm.

starved group ($p = 0.05$; Spearman rank correlation). However, this result for the 13 C starved group was based on only four abalone, which may not be reliable. A pool of the 13 C fed and starved abalone also showed a significant correlation ($p = 0.01$; Spearman rank correlation). In contrast, a pool of the 20 C fed and

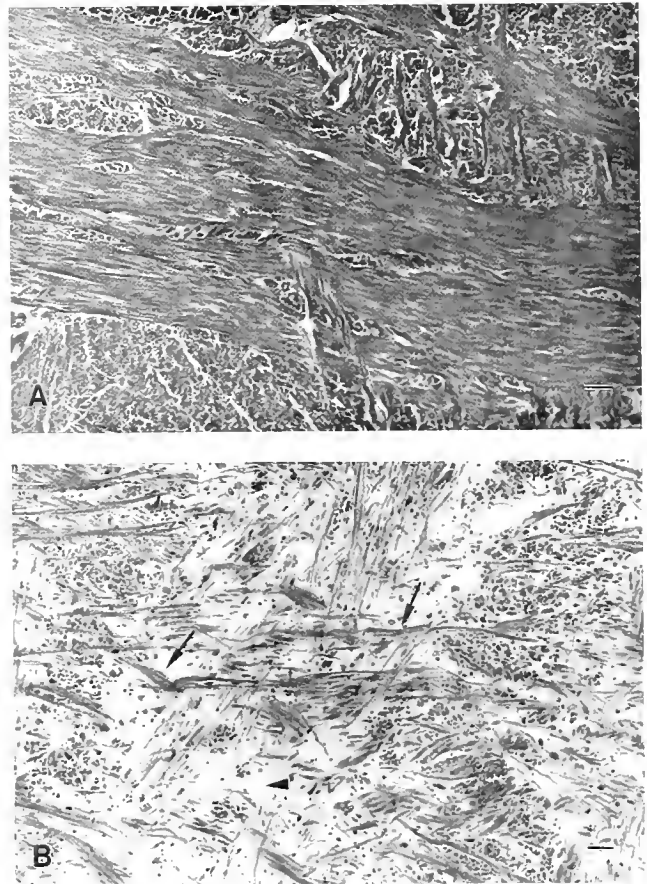


Figure 7. Photomicrograph of a cross-section through the foot muscle of two black abalone. (A) Healthy animal with dense muscle bundles in close association. (B) Animal with advanced WS. Note the severe depletion of muscle bundles (arrows) and increase in visible connective tissue (arrowhead). Hematoxylin & eosin. Bars = 50 µm.

starved abalone revealed no correlation between RLP and time to abalone death ($p = 0.44$; Spearman rank correlation).

DISCUSSION

Our field study demonstrated a broad host and geographic range of the renal coccidian in abalone from California. However, no consistent statistically significant associations between abalone condition and intensity of coccidian infection were identified. We did observe two occurrences of p values close to significance in tests of abalone condition and coccidian infection intensity in both kidneys for the pooled field data ($p = 0.07$ [Kruskal-Wallis test] and $P = 0.06$, [χ^2 test of independence]). However, as in previous studies (Friedman et al. 1993), we could not see any meaningful relationship between abalone condition and coccidian infection intensity in our data. A higher percentage of the healthy abalone had very heavy coccidian infections in both kidneys as compared with those of the very shrunken abalone (Fig. 3). A lower percentage of the healthy abalone was uninfected in both kidneys as compared with the very shrunken abalone. These results suggested that this parasite was not associated with WS and the decline in black abalone populations. Results from our laboratory study also supported this conclusion. Although testing the rank correlation between coccidian infection in the right kidney and change in abalone condition for the temperature study data was close to significant ($p = 0.07$; Spearman rank correlation test), the rank correlation was -0.16 , which was low, but negative. A negative correlation implies that a higher level of coccidian infection in the right kidney is associated with improved abalone condition, which is counterintuitive. These data provide further evidence that the coccidian is not associated with WS and appears to be nonpathogenic. Similar results of a lack of pathogenicity were reported for *P. haliotis* in red and pinto abalone (Friedman et al. 1993).

Initial observations of WS at the Channel Islands and Diablo Cove were associated with elevated water temperatures. WS was first observed on the Channel Islands after the 1982 to 1983 and 1986 to 1987 El Niños (Tissot 1991, Richards and Davis 1993). In addition, WS was observed in abalone within the thermal effluent from water used to cool the Diablo Canyon Power Plant beginning in 1988, long before the disease had spread north of the Channel Islands (Anonymous 1988, 1990). Results from our laboratory experiments described herein suggested, however, that elevated temperature was not a direct cause of WS, but accelerated the mortality of black abalone with WS. Figure 4 illustrates that mortality was greater and more rapid in animals held at 20°C relative to those held at 13°C .

Tissot (1991) hypothesized that starvation due to a loss of kelp during the 1986 to 1987 El Niño was associated with WS. However, he also observed that kelp abundance had returned to normal after the El Niño with no decrease in the prevalence of WS. Haaker et al. (1992) also observed that WS could not be reversed by feeding in a laboratory study. Our experiments revealed that at a particular temperature, both fed and starved abalone had similar survival. Abalone with WS fed on kelp until the animal reached the terminal stages of the disease, when visible atrophy of the foot muscle was easily observed (Kismohandaka et al. 1993). However, when only fed abalone were considered, our study also showed that elevated water temperature decreased survival.

These observations contrasted an observation of Young (1964),

who documented black abalone from White Point, Santa Monica Bay, with signs similar to those of WS. He attributed the poor condition of the White Point abalone to starvation and caustic pollutants released into Santa Monica Bay from a sewage outfall. Young (1964) did a reciprocal translocation experiment between White Point and Catalina Harbor, Santa Catalina Island, where kelp was abundant and no sewage was released. The translocated White Point abalone increased in body weight (15–40%) within 2 mo. Those from Catalina Harbor, in contrast, showed no weight change after 2 mo at White Point and eventually died. Young concluded that the quick reversal of abalone condition between the two locations suggested environmental causes. The reversal of an abalone's condition in Young's study indicated that the black abalone were not affected by WS.

A lack of food in our investigations did not appear to be directly associated with WS. Carefoot et al. (1993) concluded that 27 days of starvation had no debilitating effect on the overall health of the pinto abalone used in their study. Starvation did deplete glycogen reserves in the digestive gland within 6 days and in the foot muscle within 27 days and reduced hemolymph glucose levels by 50% but did not alter condition index. In an earlier study, abalone with WS were shown to deplete foot muscle glycogen before the degeneration of the foot muscle (Kismohandaka et al. 1993). Depletion of glycogen reserves was also reported in abalone with WS from a field survey (Gardner et al. 1995). This suggests that another factor such as an infectious agent may interfere with an animal's desire to eat or with its ability to digest food.

All black abalone with degeneration of the digestive gland (primarily showing one or more of the following: a decrease in abundance and necrosis of digestive diverticulae, an increase in connective tissue, or an apparent increase in the ratio of transport ducts to terminal acini within the digestive gland) had visible signs of WS (mantle retraction and foot muscle atrophy). Our laboratory study suggested that the RLP was not associated with WS, because no association was found between condition of the digestive gland and intensity of the RLP infection. Time to abalone death did not correlate with intensity of RLP infection, except in a pool of the 13°C treatments and possibly in the 13°C starved treatment. These data suggest that at lower seawater temperatures, the RLP may affect survival. However, these significant observations may be a result of small sample sizes. Only 22 animals were included in the 13°C treatment analysis, and only 4 of these were in the starved treatment. The results need to be verified with larger sample sizes. In addition, this observation was inconsistent with results from the rest of our study and those previously reported (Sommerville 1991, Tissot et al. 1991).

Field observations suggest that WS has a long incubation period before the appearance of clinical signs (e.g., digestive gland degeneration or atrophy of the foot muscle; Friedman unpubl.). Thus, it is possible that the asymptomatic animals infected with RLPs in our studies may have been in the initial and undetectable stages of WS. Alternatively, the geographic distribution of WS and the RLP may overlap but be unrelated. Physiological studies of Kismohandaka et al. (1993) indicated a decrease in food consumption and an increase in ammonia excretion in abalone with clinical WS. These data suggested that impairment of nutrient absorption may be involved in WS. Infection of digestive epithelial cells by the RLP and associated loss of secretory epithelia could alter an abalone's ability to absorb nutrients and cause the animal to wither away.

The pattern of spread of the disease, the inability to reverse WS

under optimal laboratory conditions, and its association with elevated water temperatures suggested that an infectious agent is a cause of WS. Gardner et al. (1995) recently proposed the RLP, initially observed by VanBlaricom et al. (1993), as a potential cause of WS. Gardner et al. (1995) stated that "the intensity of infection and tissue damage coincided with the signs associated with early to well-established disease" in the 25 abalone with WS and 7 abalone without WS that they examined. However, they did not provide data or statistical analyses to support this claim. Despite this, RLPs were not observed in the seven healthy abalone that they examined from Ano Nuevo Island, where WS was absent. Recently, rickettsia and rickettsiales-like bacteria have been implicated in the mortality of several marine animals: spot prawns, *Pandalus platyceros*, in British Columbia, Canada (Bower et al. 1996); coho salmon, *Oncorhynchus kisutch*, in Chile (Cvitanich 1991); and scallops, *Pecten maximus*, in France (LeGall et al. 1988). Thus, RLPs may be more pathogenic than originally suspected. These conflicting observations suggested that further investigation of a possible association between WS and this rickettsiales-like bacterium is warranted.

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PREDATOR-PREY INTERACTIONS BETWEEN THE NATICIDS *EUSPIRA HEROS* SAY AND *NEVERITA DUPLICATA* SAY AND THE ATLANTIC SURFCLAM *SPISULA SOLIDISSIMA* DILLWYN FROM LONG ISLAND TO DELAWARE

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ABSTRACT Interactions between the naticids *Euspira heros* (Say, 1822) and *Neverita duplicata* (Say, 1822) and their prey, the Atlantic surfclam *Spisula solidissima* (Dillwyn, 1817), were reconstructed from 1,300 bored shells collected at six localities from Long Island to Delaware. Both naticids show comparable site selectivity on prey valves, with 90% of complete boreholes from any one locality situated in the umbonal region, slightly posterior to the dorsoventral axis. Variation in complete borehole location in biaxial scattergrams was least for the *N. duplicata*-affected locality in Great Egg Harbor. Incomplete boreholes show a similar distribution to complete boreholes, which suggests that failure to penetrate the shell was not a consequence of poor siting of the drillhole. Modal frequency of boreholes occurs in valves between 20 and 59 mm in anteroposterior width, with a size refuge from predation at widths greater than 120 mm, although unbored specimens reached a maximum width of 160 mm. Mean borehole diameter is greatest (5.7 mm) for the *E. heros*-affected northernmost sample from Fire Island Inlet, NY, and least (3.3 mm) for the *N. duplicata*-affected Fenwick Island, DE, sample. In addition to possible taxonomic differences in radula size between naticid species, older and younger cohorts (age classes) may dominate the northern and southern naticid populations, respectively. Regressions of outer borehole diameter (OBD) on prey valve width indicate that naticids were prey size selective, although the degree of correlation varies significantly from *E. heros*-affected Barnegat Inlet ($r = 0.81$) to *N. duplicata*-affected Great Egg Harbor Inlet ($r = 0.33$) samples. High versus low correlations indicate few versus many predator-prey mismatches, i.e., oversized or undersized OBDs relative to surfclam valve width. Variation in prey size selectivity is attributed to age (size)-class dominance, or lack of, among contiguous populations of surfclams and naticids. Significantly lower correlation coefficients, based on field death assemblages relative to published statistics based on naticid predation in captivity are attributed to stochastically interrupted foraging of moonsnails on disturbance-prone exposed tidal flats versus disturbance-minimized aquarium experiments. Slopes of regression lines from *E. heros*-affected samples show a rate of increase in OBD with increasing clam prey width that is three times that of the *N. duplicata*-affected Great Egg Harbor sample. Prey effectiveness, indexed by the ratio of valves with incomplete to total attempted boreholes, is 0.01 for Fenwick Island, a surfclam population dominated by young cohorts, to 0.22 for Barnegat Inlet, a population with many old-aged individuals.

KEY WORDS: *Euspira heros*, *Neverita duplicata*, *Spisula solidissima*, naticids, surfclam, boreholes, size selectivity, site selectivity

INTRODUCTION

The surfclam, *Spisula solidissima* (Dillwyn, 1817), is a large, ovate, unornamented, fast-burrowing, shallow infaunal clam that ranges from Nova Scotia to South Carolina from the very low intertidal zone to 30-m water depth. Both the northern moonsnail *Euspira heros* (Say, 1822) and the lobed moonsnail *Neverita duplicata* (Say, 1822) (Fig. 1) prey on surfclams, drilling a counter-sunk borehole (Carriker 1981, Kowalewski 1993). Attacks are not invariably successful. Kitchell et al. (1986) successfully discriminated functional from nonfunctional perforate boreholes. In the latter case, inner borehole diameter is not large enough for insertion of the proboscis (Carriker and VanZandt 1972). Incomplete boreholes in surfclam valves (Fig. 2F) are not necessarily a consequence of a naticid attempting to drill a shell that was too thick. A large predator may have been unable to finish drilling through the shell, leaving a correspondingly large but shallow, faintly rimmed depression. Subsequently, the same individual was re-encountered by a smaller naticid, which drilled a small, complete borehole encircled by the planed-off, failed first attempt (Fig. 2J). Occasionally, a surfclam survives more than one predatory attempt, as evidenced by multiple incomplete boreholes in its valves (Fig. 2F and L). The same position on the valve may have experienced two aborted drilling attempts that left overlapping, shal-

low, incomplete boreholes (Fig. 2M). Moonsnails rarely drill a second complete, functional borehole (Fig. 2D and H) into a clam that was successfully drilled previously, but possibly survived (Kitchell et al. 1986).

Despite these uncommon outcomes, naticid boreholes in *S. solidissima* serve as evidence of predictable interactions between moonsnails and surfclams, similar to drillholes made by *N. duplicata* (formerly *Polinices duplicatus*) feeding on *Mya arenaria*, *Mercenaria mercenaria*, and *Anadara ovalis* that coexist with the Atlantic surfclam (Kitchell et al. 1981). Franz (1977) established a correlation between size of *E. heros* (formerly *Lunatia heros*) and size of bored surfclam prey based on sampled death assemblages of *S. solidissima*. A previously untested hypothesis is that predator-prey interactions with surfclams may vary between *E. heros* and *N. duplicata*, owing to different foot masses, a critical factor in the manipulation and subjugation of bivalve prey (Ansell 1960, Hughes 1985, Kabat 1990). Shell apertural or opercular area is an adequate estimator of naticid foot biomass (Kelley 1988, Rodrigues et al. 1987). Although the aperture of *E. heros* is nearly oval, that of *N. duplicata* is more comma shaped (Fig. 1). At the same shell body whorl diameter, the greater apertural area for the northern moonsnail accommodates a greater egressing foot mass than that for the lobed moonsnail.

Differences in naticid foot mass may influence siting of bore-

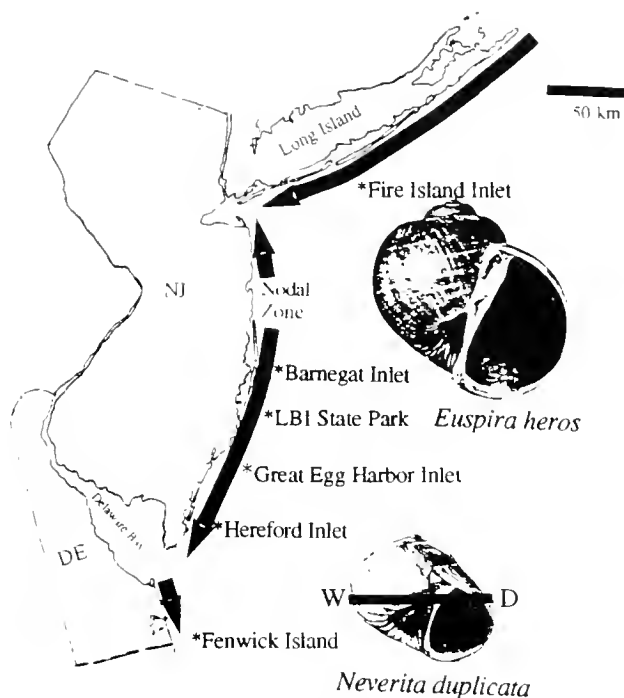


Figure 1. Location of surfclam sample sites, direction of nearshore littoral currents, and apertural view of *E. heros* and *N. duplicata*. WD, whorl diameter. Collection months in 1997 for samples are Fire Island Inlet, Jan.; Barnegat Inlet, Feb.; Long Beach Island State Park, Aug.; Great Egg Harbor, July; Hereford Inlet, July; Fenwick Island, Feb.

holes on surfclams. Because the thickness of valves protecting surfclams varies predictably both anteroposteriorly and dorsoventrally (Kitchell et al. 1981), stereotypic siting behavior functions to standardize expected drilling time costs on the same size clam prey. Stereotypic siting behavior varies among naticid species and may be concentrated on the ventral margin of the bivalve shell (Ansell 1960, Ansell and Morton 1985), near the umbonal area (Vignali and Galleni 1986, Kitchell et al. 1981, Sohl 1969, Negus 1975), or in the valve midregion (Griffiths 1981, Aitken and Risk 1988, Vermeij et al. 1989). Although the beak area of *S. solidissima* is commonly bored by both *E. heros* (Fig. 2B) and *N. duplicata* (Fig. 2H), complete boreholes are left in all regions of the surfclam (Fig. 2A, C, E, and K). However, which, if either, of these naticid species displays greater stereotypic behavior in siting of boreholes on surfclams remains to be addressed. Berg and Porter (1974) reported that *P. duplicatus* and *L. heros* bore distinctly different areas of *M. arenaria*.

Naticid gastropods are highly selective with respect to bivalve prey size (Ansell 1960, Kitchell et al. 1981, Vignali and Galleni 1986, Rodrigues et al. 1987). Kitchell et al. (1981) documented that outer borehole diameter (OBD) is correlated with predator size. Accordingly, regressions of OBD on surfclam prey valve width should result in equations (lines) with different slopes for the two naticid species if equal-sized individuals of *E. heros* and *N. duplicata* commonly prey on different sizes of surfclams. Correlation coefficients of the regressions of OBD on surfclam prey width for each naticid species should be significantly different if prey size selectivity is relaxed in either naticid species.

Prey size selection may be influenced by prey effectiveness (PE) in thwarting naticid predation. Efficiency of the predator is equivalent to the ineffectiveness of the antipredatory defense and

is indexed by the ratio of number of incomplete boreholes to combined number of complete and incomplete boreholes (Vermeij et al. 1989). Limits to prey handling (size or thickness) result in incomplete boreholes, which represent an investment of foraging time not compensated by any energetic return. Surfclams may attain a valve width in adulthood that cannot be enveloped by the largest foot of either naticid species. Surfclams also have an unusual behavioral defense, namely, the ability to leap out of the sediment on the rapid extension of their muscular foot when touched by a predator (Feder 1972), and species of this genus quickly reburrow (Stanley 1970, Alexander et al. 1993). Given their array of defenses, are surfclams any more efficient in deterrence of predatory attempts by *E. heros* relative to similar-sized *N. duplicata*? Differences should be reflected in PE values of each naticid species-surfclam interaction.

This investigation, then, addresses three questions, all of which may be related to differences in foot geometry, and associated radula size, of comparable-sized *E. heros* versus *N. duplicata*. What are the differences, if any, in: (1) stereotypy of borehole siting inflicted by each species, (2) mean size of surfclams bored by comparable-sized individuals of each species, and (3) probability of prey survival when attacked by either species.

MATERIALS AND METHODS

More than 1,300 completely and incompletely bored valves of the large, equivalved mactrid *S. solidissima* were collected from January 1997 to August 1997 from beaches near: (1) Fire Island Inlet, Long Island, NY; (2) Barnegat Inlet, NJ; (3) Long Beach Island State Park, NJ; (4) Longport Beach, Great Egg Harbor Inlet, NJ; (5) Hereford Inlet, Stone Harbor, NJ; and (6) Fenwick Island, DE (Fig. 1). Intertidal bivalve death assemblages to evaluate questions of prey site and size selectivity by naticids have been successfully used by Ansell (1960), George (1965), Negus (1975), Franz (1977), and Vignali and Galleni (1986), although selective current winnowing of smaller-sized juveniles may complicate analysis of intensity of predation on successive age classes. Surfclams from Fire Island Inlet were most likely bored by *E. heros* (Franz 1977), *N. duplicata* being reportedly rare from adjoining localities (Brinkhuis 1980). Nearshore surfclams from Barnegat Inlet were also preyed on predominantly, if not exclusively, by *E. heros*, on the basis of field observations. In contrast, the southernmost surfclam sample from Fenwick Island, DE, was preyed on by the nearshore naticid *N. duplicata*. *E. heros* is found alive only in deeper offshore habitats south of Delaware Bay. Surfclams from the shallow subtidal-intertidal area of Hereford Inlet are preyed on predominantly by *N. duplicata*. A random sample of naticid shells commingled with the surfclams included 72% ($n = 572$) and 28% ($n = 225$) *N. duplicata* and *E. heros*, respectively. (Dietl and Alexander 1995). The naticid sample from Great Egg Harbor Inlet was overwhelmingly dominated by *N. duplicata* ($n = 445$; 87% of combined sample of both naticids; Dietl and Alexander 1995). Slightly reduced salinities (~29 ppt) at embayment entrances may preclude the slightly more stenohaline *E. heros* (Michael Castagna, Eastern Shores Laboratory, pers comm.). The surfclam sample from Long Beach Island State Park was commingled with nearly coequal abundances of *E. heros* and *N. duplicata*.

Shell anteroposterior width and dorsoventral length of surfclam valves were measured with Vernier calipers to the nearest 0.05 mm. Both OBD and inner borehole diameter (IBD) were measured to the nearest 0.05 mm on complete boreholes with a micrometer-

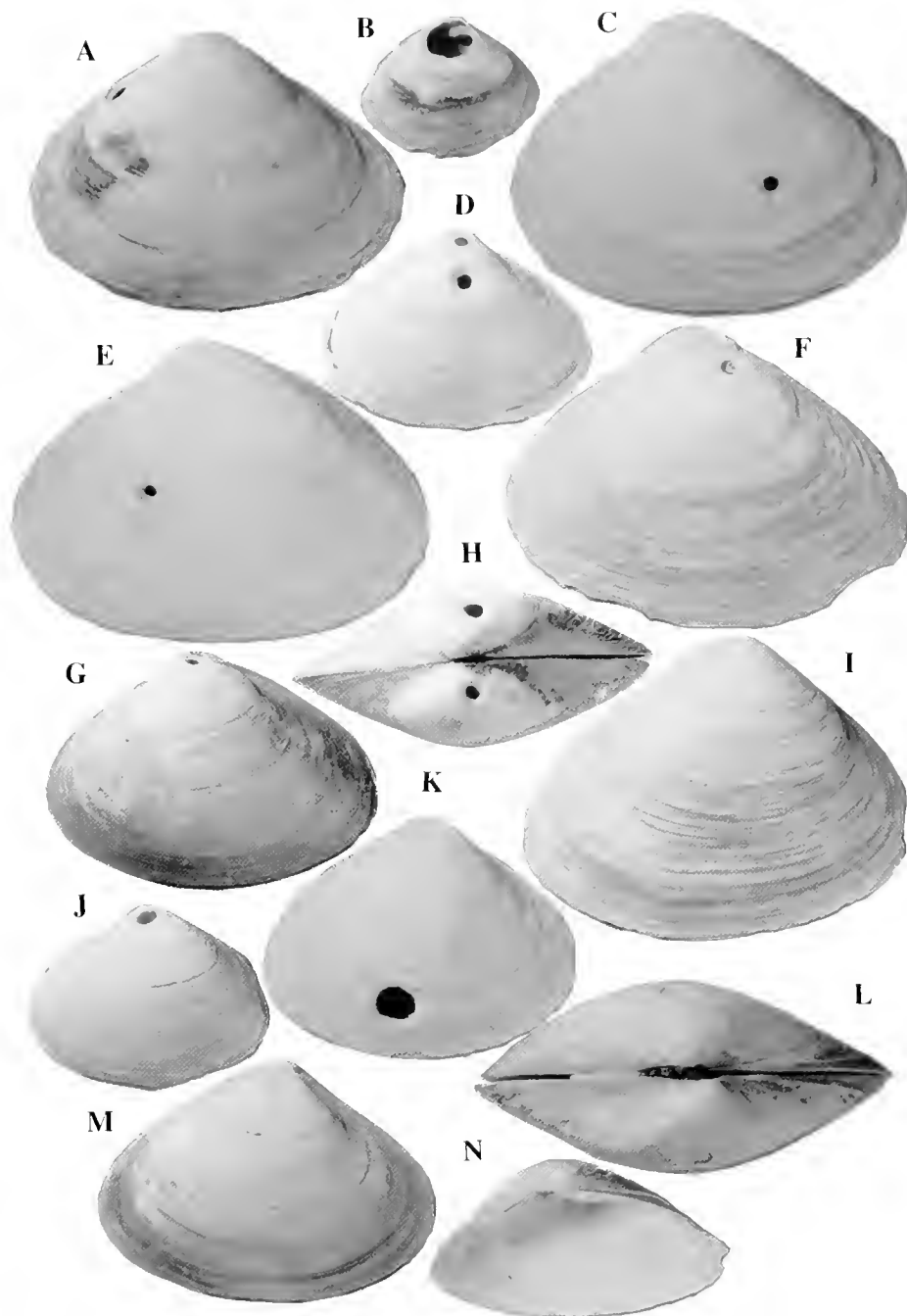


Figure 2. Incomplete and complete boreholes in valves of *S. solidissima*. (A) Posterodorsal-located complete borehole, $\times 1.2$, Fire Island; (B) oversized complete borehole in dorsal area, $\times 1.5$, Fire Island; (C) posteroventral-located complete borehole, $\times 1.0$, Hereford Inlet; (D) juxtaposed equal-sized complete boreholes in dorsal area of valve, $\times 1.0$, Hereford Inlet; (E) anterocestrally located complete borehole, $\times 1.0$, Hereford Inlet; (F) two incomplete boreholes in dorsal area of valve, $\times 0.5$, Hereford Inlet; (G) two equal-sized complete boreholes in beak area on opposing valves, $\times 1.0$, Hereford Inlet; (H) two equal-sized complete boreholes in beak area on opposing valves, $\times 1.0$, Hereford Inlet; (I) undersized incomplete borehole in dorsal area, $\times 0.3$, Hereford Inlet; (J) complete borehole within slight depression of incomplete borehole in beak, $\times 1.3$, Barnegat Inlet; (K) ventrally located complete borehole, $\times 1.2$, Fire Island; (L) equal-sized, incomplete boreholes in dorsal areas of opposing valves, $\times 1.0$, Barnegat Inlet; (M) overlapping, equal-sized, shallow, incomplete boreholes in beak, $\times 1.0$, Hereford Inlet; (N) incomplete borehole in beak, $\times 0.7$, Hereford Inlet.

calibrated eyepiece mounted on a binocular microscope. Both OBD and IBD have been shown to be a reliable index of predator size (Ansell 1960, Kitchell et al. 1981, Vignali and Galleni 1986). Ratio of IBD:OBD among complete drillholes was calculated to determine if the borehole was functional (ratio > 0.5) or nonfunctional (< 0.5), as previously discriminated by Kitchell et al.

(1986). Subsequently, rare ($< 1\%$) nonfunctional complete boreholes were excluded from procedures that involved complete boreholes.

Drilled specimens were sorted according to anteroposterior width into six, successive 20-mm-interval size classes (Fig. 3). A goodness-of-fit test determined if frequency of complete drillholes

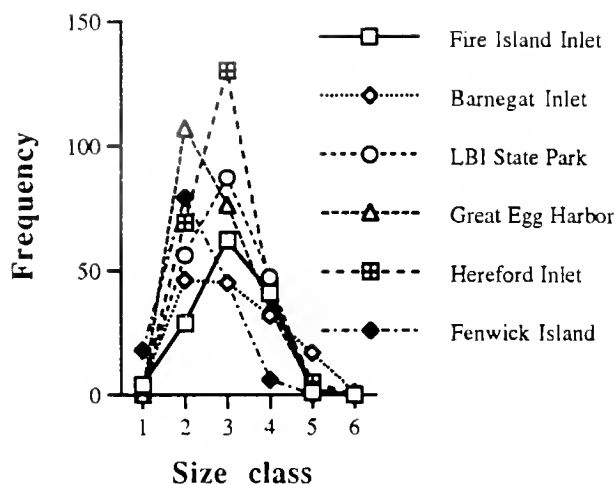


Figure 3. Frequency of boreholes in valves sorted according to prey size (width) class for each sample. Size class intervals are 1 = 1–19 mm; 2 = 20–39 mm; 3 = 40–59 mm; 4 = 60–79 mm; 5 = 80–99 mm; 6 = 100–119 mm. Null hypothesis of randomness for complete drillholes is rejected in all cases ($p < 0.001$, goodness-of-fit test).

was randomly distributed among the six prey size classes. The site of each drillhole was assigned to one of nine sectors in a grid superimposed on the valve surface (Fig. 4), following the methodology of Kelley (1988). The null hypothesis that all sectors were drilled proportional to the surface area was tested by a goodness-of-fit test following Anderson (1992). The location of complete boreholes on the valve surface was also displayed in bivariate scattergrams of the (x, y) coordinates relative to the anteroposterior (abscissa) and dorsoventral (ordinate) axes of the valve (Fig. 5), a technique used by Berg and Porter (1974), Griffiths (1981), and Anderson (1992).

However, proportional position of an incomplete borehole relative to any valve margin at the time of death of the clam is not necessarily the proportional position at the time of the unsuccessful naticid attack. An unsuccessful attempt to drill the ventral margin of a young adult would leave an incomplete borehole that would occupy an increasingly more central position with subsequent concentric shell accretion. A new methodology is introduced to compare positions of incomplete with complete boreholes. A tangent is drawn to the valve beak, producing equal umbonal angles with the dorsal margins on either side of the beak (Fig. 6). A perpendicular to the tangent divides the valve surface into anterior and posterior portions. A line is drawn from the center of complete and incomplete boreholes to the junction of the tangent and the dorsoventral perpendicular (Fig. 6C). Obtuse or acute angles formed by that line with the anterior portion of the tangent did not change with any subsequent concentric accretion of the valve after the unsuccessful attack. Angular positions of complete and incomplete boreholes were plotted on rose diagrams, and the mean angles were statistically compared by the Watson-Williams test for incomplete borehole-rich samples from *E. heros*-affected Barnegat Inlet versus *N. duplicata*-affected Great Egg Harbor Inlet (Fig. 6).

The OBD of complete boreholes was regressed on prey anteroposterior width for each sample to determine any correlation between predator and prey sizes (Kitchell et al. 1981) (Fig. 7). Slopes (B) of regression lines were statistically compared (F -test) to determine if rate of predator size (OBD) increase relative to prey size

(W) increase differed between surfclam populations (dominantly) preyed on by *N. duplicata* versus *E. heros* (Table 1). Correlation coefficients, r , were compared to evaluate flexibility in prey size selectivity for each naticid species at different localities (Table 1). Mean and variance in OBDs among surfclam samples were tested using analysis of variance/Tukey-HSD test and F -ratio test, respectively (Table 2). PE was calculated for each sample by the ratio of incomplete boreholes to total attempted predations (Vermeij 1989) (Table 3).

RESULTS

Complete boreholes are disproportionately concentrated in smaller size classes of *S. solidissima* ($p < 0.001$, goodness-of-fit test; Fig. 3). The greatest frequency of boreholes occurred in the second and third smallest prey size classes (width = 20–59 mm), whereas the largest prey size classes (width = > 100 mm) were very infrequently drilled (Fig. 3). The highest frequency of boreholes in the penultimate prey size class (width = 80–99 mm) occurred in the *E. heros*-affected sample from Barnegat Inlet (Fig. 3). Borehole site stereotypy (selectivity) is evident for both naticid predators. Sector 2, the mid-dorsal or umbonal area of the shell, hosts more than 90% of boreholes for each sample (Fig. 4). Eighty percent of incomplete boreholes are also concentrated in the second sector. Furthermore, bivariate plots of dorsoventral and anteroposterior positions of complete boreholes (Fig. 5) show tight clustering in the umbonal area (Sector 2) of valve "morphospace" for all samples. Variation in position of complete boreholes is comparable for all samples, with a few outliers in at least two of the sectors, save for the tight clustering of drillholes in the sample from Hereford Inlet (Fig. 5E). The mean angle of the complete and incomplete boreholes relative to the anterior portion of the tangent to the beak is 108° and 109° , respectively, for the Great Egg Harbor Inlet, *Neverita*-affected sample (Fig. 6B). For the *Euspira*-affected Barnegat Inlet sample, the mean angle is 107° and 108° for position of complete and incomplete boreholes, respectively (Fig. 6A). The overwhelming majority of boreholes is positioned

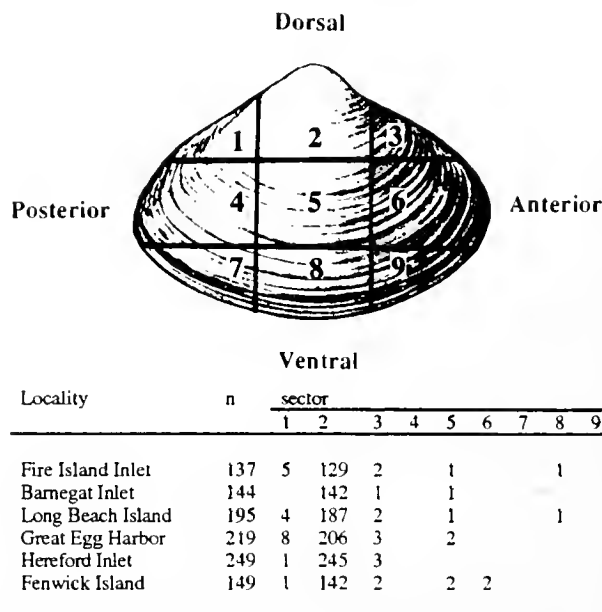


Figure 4. Frequency of complete boreholes distributed among nine sectors of a valve.

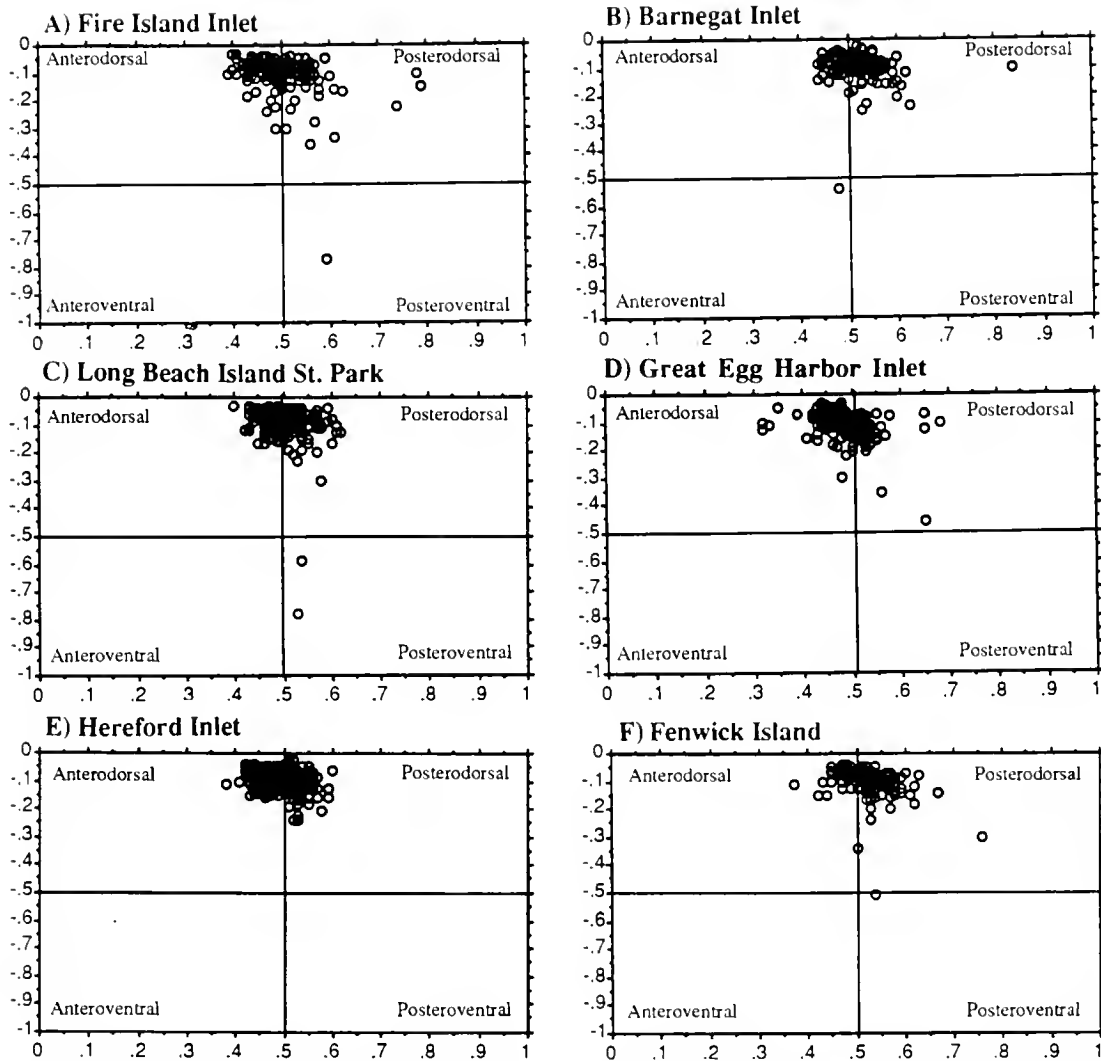


Figure 5. Distribution of boreholes measured relative to anteroposterior and dorsoventral valve axes for each sample.

slightly posterior of the dorsoventral perpendicular to the umbonal tangent in both geographic samples (Fig. 6), and no significant statistical difference is apparent between the angular position of incomplete versus complete boreholes. Although naticids were site selective on the valves, a goodness-of-fit test also shows that moon snails were not selective between left and right valves. Shells infrequently bear almost identically positioned, equal-sized complete and incomplete boreholes in opposing umbos of articulated valve (Fig. 2H and L).

Linear regressions show that naticid predator size (OBD) increased with prey size (width) for all locations (Fig. 7). A significant correlation occurred for samples suspected to have been drilled by either *E. heros* (0.65–0.81) or *N. duplicata* (0.71–0.33) (Fig. 7). Mixed naticid predator-affected samples (Long Beach Island State Park and Hereford Inlet) also show a significant correlation between OBD and prey width ($r = 0.59$ and 0.65 , respectively; Fig. 7C and E). As prey size increased from a valve width of 15 to 120 mm, the OBD drilled into the surfclams correspondingly increased from 1.0 to 9.0 mm (Fig. 7). Comparisons (F -test) of the slopes of these regression lines (Table 1) showed significant differences between the *Euspira*-affected two northern localities

(Fig. 7A and B) versus all localities south of Barnegat Inlet (Fig. 7C–F). The Fire Island Inlet and Barnegat Inlet samples have regression lines with significantly steeper slopes ($\beta > 0.060$) than those of mixed naticid predator-affected (0.048–0.055) and *Neve-rita*-affected prey samples (0.021–0.047) (Fig. 7). Furthermore, a multiple comparison Tukey-HSD test (Table 2) indicates that mean OBDs from the two northernmost sites (5.7 and 5.4 mm) are significantly greater than the mean OBD for any other locality (Table 2). Over the range of bored valve sizes common to all localities, namely, valve widths of 20–70 mm (Fig. 7), surfclams from Fire Island Inlet and Barnegat Inlet display larger mean diameter boreholes (e.g., Fig. 2B and K) versus valves of *S. solidissima* from Great Egg Harbor and Fenwick Island (e.g., Fig. 2C, D, and F). At a valve width of 70 mm, a nearshore surfclam on the Delaware Coast was most likely to be drilled by a lobed moon snail, which leaves behind a borehole with an OBD of 4.5 mm (Fig. 7F). The same size surfclam on Long Island was most likely drilled by a *E. heros*, which leaves an OBD greater than 6 mm (Fig. 7A).

Comparisons of correlation coefficients reveal that the r value for the Barnegat Inlet (0.817) sample is significantly greater than

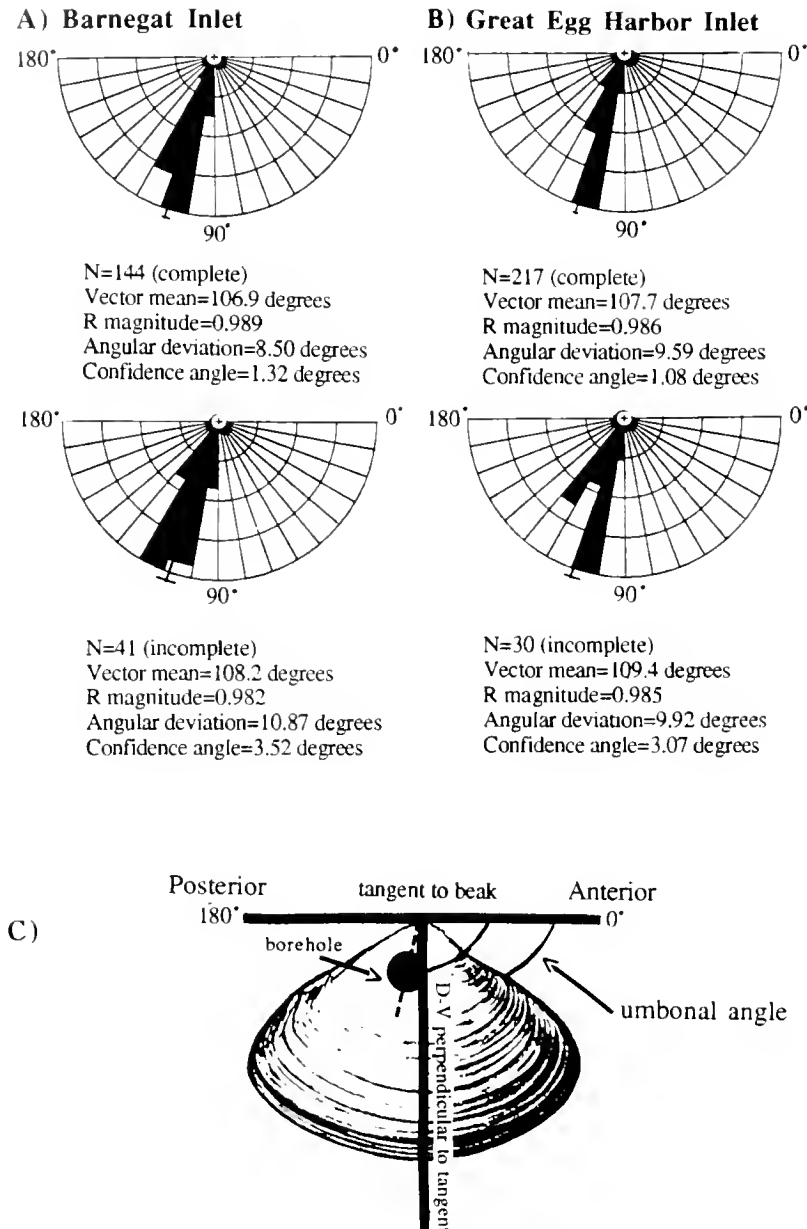


Figure 6. Angular deviation between borehole-beak axis and dorsoventral axis perpendicular to beak tangent for each complete and incomplete borehole from (A) Barnegat Inlet and (B) Great Egg Harbor Inlet. Class interval = 10°. No significant difference ($p > 0.05$) in mean angles for Panels A or B based on F -ratio in Watson-Williams test.

that for all other samples (Table 1). In contrast, the correlation coefficient for the sample at Great Egg Harbor ($r = 0.33$) is significantly lower than all other r values. Variance in OBD is also greatest and least from the Barnegat Inlet and Great Egg Harbor Inlet samples, respectively (Table 2), indicating that variance in predator size was greatest and least for these two localities, respectively.

PE ranges from 0.01 to 0.22 (Table 3). The PE is least for the surfclam sample from Fenwick Island, victimized by *N. duplicata*, which left only one incomplete borehole in the sample. This sample has the highest frequency of drilled small valves (width < 40 mm; Fig. 3) and only one drilled valve more than 60 mm in width (Fig. 7). PE is greatest for the sample from Barnegat Inlet, a surfclam population preyed on by *E. heros* (Table 3).

DISCUSSION

The high frequency of boreholes in the 20–59 mm size (width) class in all samples (Fig. 3) may reflect the demographics of predator and prey populations. If young adults numerically dominated the live population, these cohorts (age classes) should be most frequently encountered and drilled by foraging naticids, regardless of prey size preference. The absence of bored and unbored shells larger than 80 mm in the Fenwick Island sample may indicate that the live population temporarily lacked gerontic individuals. Alternatively, if the naticid population is dominated by prey size-selective young cohorts, more young adult surfclams will be drilled, which could account for the high frequency of boreholes in the 20–59 mm size classes at three of the localities (Fig. 3). In-

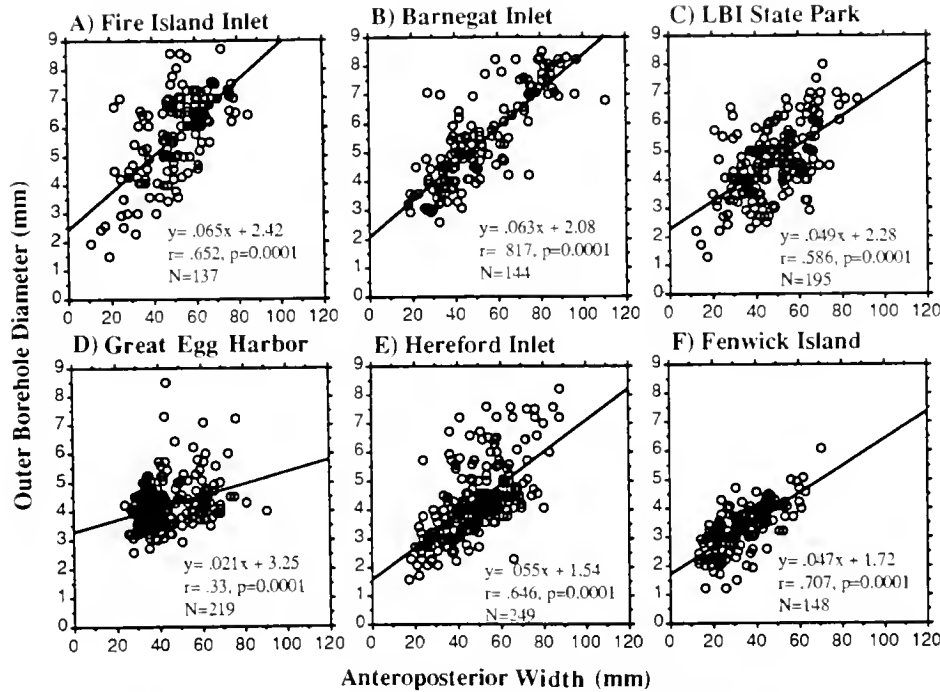


Figure 7. Regression of OBD on valve width for each sample of surfclam valves. See Table 1 for statistical comparisons of slopes and correlation coefficients of regression lines. See Table 2 for comparisons of means and variances of OBD.

terestingly, the modal size of bored shells in the Fire Island sample, namely, 40–60 mm (Fig. 3), is congruent with the 55-mm peak in the size-frequency distribution of bored specimens analyzed by Franz (1977) from the geographically proximal Rock-away Beach site. The coincidence of modes from samples 20 y apart (1997 vs. 1977) hints at stasis in the prey size class most frequently bored, but the absence of published results on yearly samples during this two-decade interval could mask yearly fluctuations in modal prey size class. The fact that half of the samples show the 20–39 mm size class and the other half show the 40–59 mm size class as the most frequently bored is probably a reflection of variation in the age-class structure of both predator and prey populations at each locality. Surfclams living near Fire Island Inlet, Hereford Inlet, and Long Beach Island State Park may be preyed on primarily by older cohorts of moonsnails relative to the naticid population structure at the other three localities (Fig. 3). Conversely, populations of *S. solidissima* from Fire Island Inlet, Hereford Inlet, and Long Beach Island State Park may be dominated by slightly older cohorts that are frequently encountered and bored by foraging moonsnails.

Regardless of which intermediate prey size class bears the highest frequency of boreholes, some surfclams may have attained a size refuge from naticid predation. No valve wider than 120 mm from any of the six collecting sites is bored, although several unbored valves measured 160 mm. However, size refuge from predation may not be invariant through time for any population of *S. solidissima*. Franz (1977) observed that some bored valves of *S. solidissima* measured 160 mm in width. During times when gerontic (very large, old-age) individuals become common in populations of *E. heros*, prey sizes that were previously too large to be enveloped by the foot of slightly smaller adults may be successfully drilled.

Although both naticid species are site selective for the dorsal area of surfclams (Fig. 4), moonsnails did occasionally penetrate the anterior, posterior, central, and ventral portions of shells (Figs. 4 and 5) from each prey population, except for the sample from Hereford Inlet. Nevertheless, no statistical test indicates that any one sample shows less stereotypy (more variation) in the location of complete boreholes versus any other samples. Likewise, incomplete boreholes are not concentrated on a different part of the valve

TABLE 1.
Comparison of slopes (β) and correlation coefficients (r) of regression lines for all six localities.

Locality	Slope	F-Ratio Value						Correlation Coefficient	Z-Statistic					
		FRI	BI	LBISP	GEH	HI	FWI		FRI	BI	LBISP	GEH	HI	FWI
Fire Island Inlet	0.065	—	0.79	49.9*	464.6*	227.7*	51.9*	0.652	—	3.13*	0.93	3.98*	0.10	0.88
Barnegat Inlet	0.063	—	—	51.4*	587.8*	19.4*	60.7*	0.817	—	—	4.35*	7.35*	3.62*	2.25*
Long Beach Island St. Park	0.049	—	—	—	241.4*	10.8*	0.81	0.586	—	—	—	3.29*	1.02	1.91
Great Egg Harbor Inlet	0.021	—	—	—	—	403.6*	186.7*	0.330	—	—	—	—	4.56*	4.92*
Hereford Inlet	0.055	—	—	—	—	—	13.8*	0.646	—	—	—	—	—	1.14
Fenwick Island	0.047	—	—	—	—	—	—	0.707	—	—	—	—	—	—

* Significant difference; $p < 0.01$.

TABLE 2.
Comparison of means and variances in OBD for all six localities.

Locality	Mean OBD (mm)	q Value in Tukey Test						Variance of OBD (mm)	F-Ratio Value					
		FRI	BI	LBISP	GEH	HI	FWI		FRI	BI	LBISP	GEH	HI	FWI
Fire Island Inlet	5.7	—	3.25	16.29*	16.20*	11.20*	23.55*	2.25	—	1.10	1.53*	3.35*	1.44*	3.29*
Barnegat Inlet	5.4	—	—	13.82*	12.89*	7.91*	20.69*	2.48	—	—	1.69*	3.69*	1.59*	3.62*
Long Beach Island St. Park	4.7	—	—	—	0.48	—	5.36*	1.47	—	—	—	2.19*	1.06	2.15*
Great Egg Harbor Inlet	4.2	—	—	—	—	4.98*	10.30*	0.67	—	—	—	—	2.33*	1.02
Hereford Inlet	4.2	—	—	—	—	—	14.18*	1.56	—	—	—	—	—	2.28*
Fenwick Island	3.3	—	—	—	—	—	—	0.68	—	—	—	—	—	—

* Significant difference; $p < 0.01$.

from complete boreholes (Fig. 6). The fact that both complete and incomplete boreholes are sited more than 90 and 80%, respectively, in the umbo (Figs. 4 and 5) and slightly posterior to the dorsoventral axis (Fig. 6) of shells of each sample indicates that incomplete boreholes are not the consequence of poor siting on the valve surface in the attempted predation by either naticid species.

The thickness of a surfclam valve probably did not cause either naticid species to abort its predatory effort, provided the foot could envelop the valve. Numerous mismatches evident from the regression of OBD on prey width (Fig. 7) indicate that many undersized naticids successfully penetrated unpredictably large valves at each sample site (e.g., Fig. 2G). Similarly, Ansell (1960) rejected the concept that incomplete boreholes in *Venus striatula* were the consequence of prey too thick to be drilled. In aquarium-bound experiments, naticids commonly required more than 25 h to bore slightly thicker clam species, i.e., *M. mercenaria*, that coexist with *S. solidissima* (Kitchell et al. 1981). If naticids can penetrate hard-shell clams thicker than any surfclam, then there is no mechanical limit to drilling surfclams (Kitchell et al. 1981). Indeed, Franz (1977) reported the rare occurrence of a 160-mm-wide drilled shell of a surfclam. However, the large girth of some old-age shells of *S. solidissima* may present a manipulative limit to shell subjugation by the naticid foot. Similarly, Kitchell et al. (1981) experimentally demonstrated that the largest shells of *M. arenaria* could not be manipulated by the foot of *N. duplicata*, despite the fact that the very thin shells of the softshell clam would otherwise be vulnerable to penetration.

Incomplete boreholes indicate that the foot was large enough to envelop the surfclam and start the drilling process. However, given the extensive time (i.e., >day) required to penetrate large-size clam shells (Kitchell et al. 1981), incomplete boreholes in surfclams most probably represent outcomes of stochastic abiotic and biotic events interrupting the drilling process. Many naticids, including *E. heros* (Vencile and Beale 1997), seized prey when foraging on emergent intertidal flats (Hughes 1985). Two successive tidal cycles could provide ample exposure time for interruptions of the drilling process by aves and intertidal horseshoe crabs, or even conspecific, foraging moonsnails, not to mention abiotic disturbances. Moonsnails commonly show shell repairs from unsuccessful peeling attacks by portunid crabs (Dietl and Alexander in press). Additionally, escape tactics by *S. solidissima* (Feder 1972) may have frequently interrupted the drilling process.

Significant differences in the mean and variance of OBD, and the regressions (correlation coefficients, slopes) of OBD on prey width among the six localities (Tables 1 and 2), are attributed to two primary factors. The first is the numerical dominance, codominance, or exclusion of one naticid species, either *E. heros* or *N.*

duplicata, among the predator populations. Second, and no less important than the first, is the variation in the demographics of both the predator and the prey populations at each locality. The fact that the two northernmost surfclam samples, Fire Island Inlet and Barnegat Inlet, have significantly greater mean OBD (5.7 and 5.4 mm) relative to other samples (Table 2), could be a manifestation of predator populations overwhelmingly dominated by *E. heros*, which has a larger foot mass (and radula?) at the same body whorl diameters as *N. duplicata*. Samples affected primarily, if not exclusively, by *N. duplicata*, namely, Great Egg Harbor Inlet and Fenwick Island, have mean OBD of 4.2 and 3.3 mm, respectively, the smallest values among the samples (Table 2). However, differences in the demographics of the naticid populations could produce the same effects as different radular architectures between the two predators. The significantly smaller mean OBD in the samples from Fenwick Island and Great Egg Harbor Inlet may be the consequence of predation by predominantly younger cohorts of *N. duplicata*, whereas larger mean OBDs in samples from Fire Island Inlet and Barnegat Inlet may be a product of predation by predominantly older cohorts of *E. heros* (Table 2). Thus, significant decreases in mean OBD in surfclams latitudinally from Fire Island Inlet to Fenwick Island may be a consequence of replacement of *E. heros* by *N. duplicata* and/or contiguous populations of naticids that are dominated by progressively younger cohorts from north to south.

Greater positive slopes of regression lines for the two north-

TABLE 3.
Mean efficiency of antipredatory defense (PE) in deterrence of predation by drilling (PE = number of incomplete drillholes/total attempted drillholes) for *S. solidissima*.

Taxon	Locality	Complete	Incomplete	PE
<i>Spisula solidissima</i>	Fire Island Inlet	137	14	0.09
	Barnegat Inlet	144	41	0.22
	Long Beach			
	Island State			
	Park	196	32	0.14
	Great Egg Harbor	219	32	0.13
	Hereford Inlet	296	42	0.12
<i>Mactra chinensis</i>	Fenwick Island	148	1	0.01
	Abashiri City,			
	Japan*	36	7	0.16
<i>Pseudocardium sachalinense</i>	Tsukushikoi,			
	Japan*	20	31	0.61

* From Vermeij et al. 1989.

ernmost samples (Fig. 7; Table 1) may also be a manifestation of predation on surfclams by *E. heros*. Northern moonsnails may show allometric growth between their radula and foot mass, the former increasing in size at a faster rate than the latter, whereas *N. duplicata* may not show the same allometric growth relationship between these two morphologic characters. Accordingly, and assuming prey size selectivity, valves of successively larger prey size classes would bear boreholes that increase in size at a greater rate; hence, a steeper slope in the regression, if *E. heros* rather than *N. duplicata*, is the predator. The sample from Barnegat Inlet and Fire Island Inlet show statistically greater mean OBD versus all other samples (Table 2). Alternatively, if the predator population is dominated by young adults with little variability yet developed in the size of their radula, variance of OBDs drilled into the surfclams will be restricted regardless of affected prey sizes. In such a predator-prey system, the resulting regression line would be "nearly horizontal" because OBDs would vary insignificantly with increasing or decreasing size of drilled prey. This possibility may account for the low regression slope ($\beta = 0.021$; Fig. 7) in the Great Egg Harbor Inlet sample, which also has the smallest variance (0.67 mm) in OBDs (Table 2). Similarly, a predator population dominated by old-age naticids would correspondingly leave behind mostly similar-sized OBDs among the various prey size classes attacked. The consequence, statistically, would be a low slope to the regression of OBD on valve prey width.

The sample from Barnegat Inlet shows the highest degree of predator:prey size correlation, an r value significantly greater than the other five locations (Fig. 7; Table 1). Eschewing sampling bias, one possible explanation is that the naticid predator at Barnegat Inlet, most probably *E. heros*, was very selective. In a deterministic model, small predators usually foraged until they encountered readily manipulated prey, and larger predators foraged until they maximized energetic gain for their drilling effort by subduing a large surfclam. Such a foraging strategy increases predator fitness by maximizing the net rate of energy return per unit foraging time according to the experimental optimizing models of Kitchell et al. (1981) and Rodrigues et al. (1987). Such an energy maximization model would suggest that the population of *E. heros* at Barnegat Inlet is more selective among available prey sizes than the population of *N. duplicata* preying on surfclams near Great Egg Harbor Inlet, given the significantly lower r values for the sample from this locality (Fig. 7D; Table 1).

Once again, demographics of the predator and prey populations at each geographic locality may greatly influence the correlation between predator and prey sizes. For predator-prey size correlation to be documented, a range of potential predator sizes must have the opportunity to forage on a range of potential prey sizes. If only a few predator cohorts (age classes), either young or old, dominate the naticid population, variation in OBD in prey valves will be correspondingly limited. Any regression involving OBD as the dependent variable (ordinate on the y-axis) would be more tightly clustered, influencing the correlation coefficient, than if a broad range of predator sizes affected a given surfclam population. However, the range in OBDs, 1–9 mm for Fire Island Inlet and Barnegat Inlet (Fig. 7), suggests that several age classes of naticids are represented at these localities. Variance in the size of boreholes in naticid shells also indicates that a similar distribution of age classes of moonsnails is size selective in their confamilial predation along the southern New Jersey Coast (Dietl and Alexander 1995). Although the sample from Barnegat Inlet shows the highest degree of correlation between predator and prey size ($r = 0.81$),

the Fire Island Inlet sample, also victimized by *E. heros*, shows a degree of correlation ($r = 0.65$) comparable to that for the southernmost samples preyed on by *N. duplicata* (Table 1). On the basis of this comparison, the northern moonsnail was not invariably more size selective in its predation on surfclams than the lobed moonsnail.

Despite significant correlations between predator and prey sizes (Fig. 7), a stochastic model can account for much of the interaction between either *E. heros* or *N. duplicata* and *S. solidissima*. In such a model, the most profitable prey when encountered are always selected, but if relative abundance or encounter rates with the most profitable prey are low, less profitable prey are included in the diet of the predator (Hughes 1980). Predator-prey size mismatches apparently occurred frequently, given the low r^2 value (0.11–0.50) in five of the six samples (Fig. 7). In biological terms, the difference between the r^2 value and perfect correlation ($r = 1.0$) in regressions indicates the degree to which naticids successfully drilled larger (Fig. 2H) or smaller (Fig. 2B) prey than predicted by deterministic models of optimal foraging (Kitchell, et al. 1981). In the case of the Great Egg Harbor Inlet sample with the extremely low r^2 value (0.11), 89% of the variation in OBD cannot be explained by corresponding changes in the size of the drilled prey. Laboratory-based deterministic models may indicate which prey sizes are optimal for a given sized naticid predator based on cost-benefit curves (Kitchell et al. 1981, Rodrigues et al. 1987), but prey selection by either *E. heros* or *N. duplicata* in intertidally exposed habitats is, to a considerable degree, locally opportunistic, given the high variation in predator-prey size match-ups at some localities (e.g., Fig. 7D). If the predator encounters a potential prey surfclam, and if the foot can envelop the prey and drill it, the surfclam is likely to be consumed by the naticid.

Fortuitous encounters may result in rejection of prey by the predator if the surfclam is too small or too large. Subjugation of a clam by a naticid begins with the moonsnail repeatedly crawling around the partially exposed clam at the sediment surface, after which, the moonsnail then covers the clam with a copious pedal mucus. Enveloped shells may be dragged around on the sediment surface, and large, tenuously held prey may become dislodged from the foot's less-than-secure grip (Hughes 1985; see also review of the process by Kabat 1990). If this subjugation process also applies to naticid-*S. solidissima* interactions, the potential prey could be rejected during encirclement or mucous coating, or inadvertently released during towing on, or digging beneath, the sediment surface. Esurient naticids, foiled in their attempt to consume optimal size prey, may subsequently, frequently, settle for less optimal size prey. Given the perturbations in the intertidal habitats, vagaries that are, understandably, intentionally precluded in laboratory experiments, it is not surprising that correlation coefficients in regressions of OBD on prey width from some native habitats may be significantly lower ($r = 0.330$; Fig. 7D) than that generated experimentally ($r = 0.63$, *Polinices duplicatus* on *Anadara ovalis*, a coinhabitant of the upper subtidal with surfclams (Kitchell et al. 1981). In cannibalistic experiments with *N. duplicata*, regression of OBD on conspecific prey size, i.e., body whorl diameter, showed a very significant correlation ($r = 0.89$; Kitchell et al. 1981). However, field data on naticid predation on moonsnails produced $r = 0.69$ and 0.71 for *N. duplicata* and *E. heros*, respectively (Dietl and Alexander 1995).

The Fenwick Island sample shows very low PE in deterrence of successful predation, with only one incomplete borehole (Table 3), but this low PE may be a reflection of a surfclam population

dominated by young cohorts with thin shells rather than the efficiency of *N. duplicata*. The Barnegat sample shows the highest PE (0.22), and this sample is from a locality primarily affected by *E. heros* (Table 3). It also has the most specimens over 120 mm in width (Fig. 7), and the surfclam population may be dominated by older, larger, thicker cohorts. Similarly, a sample of *Pseudocardium sachalinense* with shell architecture resembling *S. solidissima* displays a PE of 0.61 (Table 3). More than 60% of specimens of this Japanese mactrid species were more than 75 mm wide, (maximum = 120 mm) (Vermeij et al. 1989), suggesting a population also dominated by older-age individuals. Samples from Fire Island Inlet and Great Egg Harbor Inlet were preyed on predominantly by different naticids, yet the PE values are comparable (0.09

and 0.13, respectively; Table 3). There is no unequivocal evidence that PE is better against one or the other naticid species. However, PE may be greatly influenced by predator and prey population demographics. Surfclams populations dominated by young (or old) age classes and preyed on by naticids dominated by older (or younger) cohorts may experience fewer (or more) unsuccessful attempts.

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THE MICROPREDATORS OF SETTLING AND NEWLY SETTLED QUEEN CONCH (*STROMBUS GIGAS* LINNAEUS)

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ABSTRACT Minute settlers to soft-bottom marine communities are potentially vulnerable to a variety of micropredators. Although these micropredators shape the postsettlement community structure of their benthic prey, they are not often well identified. In the laboratory, we tested species from 10 families of polychaetes, 8 families of crustaceans, 4 families of mollusks, and 2 families of fishes for their ability to kill newly settled queen conch (*Strombus gigas* Linnaeus). This species is a commercially important Caribbean gastropod that produces pelagic larvae that settle onto the benthos at ~1 mm shell length. All micropredators were collected from conch nursery areas. Conch kills were made by calappid, portunid, majid, and hermit crabs; alpheid and palaemonid shrimps; panulirid lobsters; file fish; and five families of polychaete worms (glycerids, nereids, sigalionids, spionids, and syllids). Species from five families of polychaete worms (dorvilleids, glycerids, lumbrinerids, nereids, and phyllodocids) also killed competent conch veligers. All of the species that killed conch veligers, and most of those that killed newly settled conch, had not been documented as conch micropredators before this study, and they probably have a significant effect on recruitment. Given the relatively high densities known for some micropredator species living in queen conch nursery areas, the effects of predation-induced mortality on the very earliest life stages are an important consideration in studies of queen conch population dynamics.

KEY WORDS: benthos, crustacean, fish, gastropod, polychaete, predation

INTRODUCTION

Minute settlers to soft-bottom marine communities are potentially vulnerable to a variety of micropredators. These micropredators can play an important role in structuring the postsettlement community by consuming potential recruits (Thorson 1966, Woodin 1976, Stoner 1990, Osman et al. 1992, Gosselin and Qian 1997), even to the point of eliminating them (Osman and Whitlatch 1995).

The objective of this study was to test a wide range of species for their ability to kill newly settled queen conch *Strombus gigas* Linnaeus and competent queen conch larvae. The queen conch, an herbivorous marine gastropod that produces pelagic larvae, has been severely overfished during the past 25 y (Appeldoorn 1994). Despite approximately 230 scientific articles written on this species (Acosta 1994, Stoner 1997), studies on the very early life history of queen conch are limited because they settle at ~1 mm in shell length (Davis 1994) and are difficult to find.

Although they probably have a significant effect on early benthic conch survivorship, specific micropredators are not well identified. In this study, we determine which species have the potential to affect the survivorship of recruits by consuming settling and newly settled individuals. Micropredators representing 22 invertebrate families and 2 fish families were tested after collection from queen conch nursery areas.

METHODS

Predation experiments were conducted in the laboratory on Lee Stocking Island, in the Exuma Cays, Bahamas, during May to July

1993. All experimental conch were hatchery reared and purchased from the Caicos Conch Farm in the Turks and Caicos Islands, British West Indies. Transit time to our laboratory was less than 15 h.

Potential micropredators were sorted by hand from sediment and detritus (senescent blades of the seagrass *Thalassia testudinum* Koenig) collected from Shark Rock, a well-studied conch nursery in a shallow seagrass meadow near Lee Stocking Island. Shrimps and spiny lobster were captured with collectors made from polyvinyl chloride frames that were anchored above the substrata and that held vertical racks of fibrous material (Heatwole et al. 1991). Some shrimps were also collected with otter trawls towed by small boat over the nursery.

Ultimately, 10 families of polychaetes, 8 families of crustaceans, 4 families of mollusks, and 2 families of fishes were represented in our testing. Polychaetes were considered as potential predators because during an earlier study (Davis and Stoner 1994), the shells of some competent and newly metamorphosed conch were found empty after 24 h of exposure to sediment that contained only polychaetes.

Tests with invertebrate predators were conducted in Petri dishes (diameter = 37 mm, height = 11 mm), in round, white, flat-bottom polyethylene containers (diameter = 10.5 cm, height = 5.5 cm deep), or in buckets (19 L), depending on predator size. All test containers were filled with seawater, which was changed daily. A layer of fine sand (200–300 µm) was put on the bottom of containers with polychaete predators because some species require sediment for locomotion and the building of mucous tubes

TABLE 1.
Results of tests in which conch were offered to 27 different potential predator species.

Predator Type	Characteristic Measured	Predator Size Range (mm) (n)	Conch Size (mm) (n)	Conch Range Consumed (mm) (n)	Conch Status
Crustaceans					
Diogenidae					
<i>Dardanus</i> sp. in <i>Cerithium</i> sp. shells	SL of <i>Cerithium</i>	16–17 (10)	1.6–2.1 (10)	None	Three live conch with AB
<i>Petrochirus diogenes</i> Linnaeus	CW	6.5–18 (8)	Small: 3.7–4.0 (8) Large: 26–37 (7)	3.9 (1) None	SDC entirely gone
Calappidae					
<i>Calappa gallus</i> (Herbst)	CW	28, 64 (2)	4.4, 28 (2)	4.4, 28 (2)	SDC cut in half
Majidae					
<i>Macrocoeloma camptoceran</i> (Stimpson)	CW	11 (1)	1.7, 3.7 (2)	None	Both live conch with AB
<i>Macrocoeloma trispinosum</i> (Latreille)	CW	10 (1)	2.0, 3.7 (2)	None	Both live conch with AB
<i>Mithrax</i> sp.	CW	5.9 (1)	1.5, 3.6 (2)	None	One live conch with AB
<i>Mithrax sculptus</i> (Lamarck)	CW	8.7 (1)	2.0, 4.4 (2)	None	Live
<i>Pitho</i> sp.	CW	1.7 (1)	2.0 (1)	None	Live
<i>Pitho aculeata</i> (Gibbes)	CW	2.5–12 (6)	1.4–4.4 (6)	1.7, 1.8 (2)	Two live conch with AB, SDC were crushed
<i>Pitho unisodon</i> (von Martens)	CW	8.4, 12 (2)	1.6, 3.6 (2)	1.6 (1)	SDC was crushed
<i>Pitho lherminieri</i> (Schramm)	CW	3.1–12 (9)	1.5–5.1 (10)	1.5 (1)	Six live conch with AB; 1 live conch with SD; SDC was crushed
Portunidae					
<i>Portunus spinimanus</i> Latreille	CW	5.1–34 (11)	1.6–27 (17)	1.6–27 (11)	Three live conch with AB; SDC were crushed, peeled, or otherwise damaged
Alpheidae					
<i>Alpheus viridari</i> (Armstrong)	CL	5.8–7.0 (3)	1.7–3.7 (4)	1.7, 1.9 (2)	One live conch with AB; SDC were crushed
<i>Synalpheus</i> sp.	CL	8.1 (1)	1.7 (1)	1.7 (1)	SDC with AB
Penaedidae					
<i>Metapanacopsis smithi</i> (Schmitt)	CL	8.1–13.1 (9)	2.0–4.6 (18)	None	One live conch with AB
Palaemonidae					
<i>Periclimenes americanus</i> (Kingsley)	CL	2.4–10.5 (8)	1.4–2.0 (8)	None	Live
<i>Periclimenes</i> sp.	CL	8.9–12.5 (18)	1.2–2.2 (18)	1.2–1.5 (4)	SDC were crushed
<i>Pontonia</i> sp.	CL	5.1 (1)	1.4 (1)	1.4 (1)	SDC intact
Palmuridae					
<i>Panulirus argus</i> (Latreille)	CL	5.2–6.1 (10)	1.2–4.1 (10)	1.2–4.1 (8)	SDC of 7 conch were crushed, 1 was peeled
Mollusks					
<i>Octopus briareus</i> Robson	HL	4.5, 8.8 (2)	4.4–29 (4)	None	Live
<i>Murex pomum</i> Gmelin	SL	62–77 (9)	4.0–30 (10)	None	Live
<i>Volvarna lactea</i> Kiener	SL	5.1, 6.1 (2)	1.9 (2)	None	Live
<i>Polinices lacteus</i> Guilding	not measured	(1)	2.1–4.4 (2)	None	Live
Fishes					
Monacanthidae					
<i>Monacanthus ciliatus</i> Mitchell	TL	38–44 (8)	4.0 (25)	4.0 (14)	Only conch foot and eye stalks were eaten without damage to shells
Tetraodontidae					
<i>Sphoeroides splengeri</i> Bloch	TL	30, 60 (2)	4.0 (25)	4.0 (1)	Twenty-five conch offered to all 3 tetraodontids at same time; only conch foot and eye stalks were eaten
<i>Canthigaster rostrata</i> Bloch	TL	46 (1)			
Echinoderms					
Ophiuroids					
	CDD	3.9–9.3 (8)	1.6–2.0 (8)	None	

Observations of conch status were made after 24 and 48 h, and, in some cases, after 72 h. For characteristic measured, CDD, central disk diameter; CL, carapace length; CW, carapace width; HL, head length; TL, total length. For conch status, AB, aperture breakage; SD, spire damage; SDC, shell(s) of each conch. All conch measurements are for SL.

TABLE 2.

Results of predation experiments when newly settled conch (1.2–2.1 mm SL) were offered to 16 different polychaete species (8 families).

Species	n	Head Width (mm)	Body Length (mm)	Conch Size (mm)	Hours	Conch Status
Dorvilleidae						
<i>Dorvillea sociabilis</i> Webster	1	0.425	7.7	1.4	48	Live
<i>Schistomeringus rudolphi</i> delle Chraie	2	0.325–0.375	9.6–11.9	1.6–1.7	72	Live
Eumecidae						
<i>Lysidice ninetta, collaris</i> Audouin & Milne-Edwards	2	0.700–0.850	17.4–18.3	1.4–1.5	48	Live
Glyceridae						
<i>Glycera tessellata</i> Grube	2	0.350–1.250	14.5–31.0	1.2–1.7	72	One live; 1 killed
Lumbrineridae						
<i>Lumbrinaris latreilli</i> Audouin & Milne-Edwards	1	0.950	39.7	1.7	72	Live
Nereidae						
<i>Ceratonereis mirabilis</i> Kinberg	5	0.925–1.075	10.6–26.5	1.3–2.1	48/72	Three live with AB; 1 killed at 24 h, shell empty; 1 killed at 72 h with AB
<i>Nereis (Nereis) false</i> Quatrefages	1	1.050	9.6	1.4	72	Live with slight AB
<i>Nereis (Nereis) idisei</i> Grube	1	0.875	23.3	1.5	72	Live with AB
<i>Nereis (Nereis) riisei</i> Grube	1	0.400	7.6	1.9	72	Live
<i>Platynereis dumerilii</i> Audouin & Milne-Edwards	1	0.325	4.3	1.7	72	Live
Paraonidae						
<i>Paraonis gracilis, oculata</i> Hartman	2	0.200–0.325	3.5–6.6	1.5–1.6	72	Live
Sigalionidae						
<i>Shenelais boa</i> Johnston	3	0.450–0.550	7.8–14.2	1.4–1.7	48/72	One live; 1 killed at 48 h; 1 killed at 72 h
Spionidae						
<i>Minuspio cirrifer</i> Wiren	1	0.25	9.5	1.8	48	Live
<i>Minuspio polybranchiata</i> Fauvel	1	0.3	11.1	1.7	48	Live
<i>Prionospio fallax</i> Soderstion	1	0.25	8.3	1.8	72	Killed
Syllidae						
<i>Exogone dispar</i> Webster	1	0.3	2.6	1.4	72	Killed

n is the number of individual polychaetes of a particular species that were each offered one conch. A range for polychaete head and body measurements is given. Hours is the number of hours at which the final observation of conch status was made. AB, aperture breakage.

(Fauchald and Jumars 1979). Prior to use, sand was dried at 60°C to kill any other potential predator and prey. Fishes were tested on wet tables with flow-through seawater.

After potential predators had been starved for 24 h or more, one newly settled conch was introduced into each test container. After 24 h, the status of the conch in each container was noted. If the conch was still alive, diatom food was added, and its status was observed again after at least one, and sometimes two, more 24-h periods.

Most ($n = 179$) conch prey offered to nonpolychaete predators were ≤ 5.0 mm shell length (SL) (Table 1), or approximately 2–30 days in postmetamorphic age. Seven slightly larger conch prey (5.1–10 mm SL) were offered to some majid and portunid crabs. Only 21 conch prey were ≥ 13 mm SL (range, 13–37 mm SL); these were offered to hermit, calappid, and portunid crabs and to octopus and murex.

Small conch, ranging in SL from 1.2 to 2.1 mm, were offered to polychaetes in polyethylene containers that were kept in an incubator at 28°C with a photoperiod of 12 h of light and 12 h of dark. Competent conch veligers were also offered to polychaetes to determine their ability to attack presettlement prey in the water column. When these veliger tests were conducted, five control containers, each with 10 veligers, were also monitored for survivorship. All predators were measured when the experiment took

place, except for polychaetes, which were preserved and measured during final identification.

RESULTS

Both of the calappid crabs tested killed the conch that they were offered, cutting the conch shells in half. Portunid crabs killed 65% of the 17 conch offered to them (Table 1). Of the eight majid crab species tested, only those of the genus *Pitho* made kills. Of the two species of hermit crabs tested, only *Petrochirus* made a kill, whereas *Dardanus* damaged the apertures of three conch but killed none. Alpheid shrimps (two species) killed 60% of the conch offered, palaemonid shrimps killed 19%, and penaeid shrimps killed none. Spiny lobster (*Panulirus argus*) and file fish (*Monacanthus ciliatus*) were also significant predators, killing 80 and 56% of the conch, respectively. Most of the conch (86%) killed by crustaceans were killed within 24 h. No kills were made by molluscs or brittle stars.

Five of the 16 tested polychaete species killed small conch (Table 2); 8 of the 13 tested species killed competent veligers (Table 3). Glycerids and nereids killed both small conch and veligers. Nereids also broke the shell apertures of killed veligers and small conch, but two small conch remained alive after such damage. Dorvilleids and lumbrinerids killed veligers but not small juvenile conch, and syllids killed small conch but not veligers.

TABLE 3.

Results of predation experiments when 10 competent *Strombus gigas* veligers were offered to 13 different polychaete species (8 families).

Species	n	Head Width (mm)	Body Length (mm)	Veliger Status
Dorvilleidae				
<i>Dorvillea sociabilis</i> Webster	1	0.40	11.0	10% killed; 50% meta
Eunicidae				
<i>Eunice websteri</i> Fauchald	1	0.65	16.5	All live; 30% meta
Glyceridae				
<i>Glycera tessellata</i> Grube	2	0.35–0.88	13.7–36.5	60–90% killed; 10–40% meta
Lumbrineridae				
<i>Lumbrinaris latreilli</i> Audouin & Milne-Edwards	4	0.70–1.12	23.3–65.6	100% killed in 2 tests; 0 and 10% killed in 2 tests; 10–20% meta
Nereidae				
<i>Ceratonereis mirabilis</i> Kinberg	2	0.75–0.88	12.2–16.6	10–30% killed (some AB); 30–40% meta
<i>Nereis (Nereis) greyi</i> Pettibone	1	0.78	16.6	All live; 10% meta
<i>Nereis (Neutheis) succinea</i> Frey & Leuchart	1	0.45	6.2	10% killed
<i>Platynereis dumerilii</i> Audouin & Milne-Edwards	2	0.72–0.80	9.4–14.0	10–20% killed (1 AB, 1 crushed); 30–90% meta
<i>Ceratonereis mirabilis</i> Kinberg	1	0.88	11.2	50% killed (most AB); 40% meta
Paraonidae				
<i>Paraonis gracilis</i> Tauber	1	0.50	11.6	All live; 10% meta
Phyllodocidae				
<i>Phyllodoce madeirensis</i> Langerhans	1	0.52	2	90% killed
<i>Phyllodoce (Nereiphyta) fragilis</i> Webster	1	0.25	6.3	All live
Syllidae				
<i>Syllis (Typosyllis) prolifera</i> Krohn	1	0.25	6.3	All live

n is the number of individual polychaetes of a particular species that were each offered 10 veligers. A range for polychaete head and body measurements is given. Veliger status was recorded after 24 h. AB, shells with aperture breakage; meta, live veligers that underwent metamorphosis during the experiment.

Sigalionids and spionids were only tested for small conch (Table 2), and there were kills made by each family. Phyllodocids were only tested with veligers (Table 3), and one species killed 90% of the veligers offered. Eunicids and paraonids made no kills of either veligers or small conch. Two individual, unidentified nemerteans each killed a 1.4-mm conch in less than 24 h.

None of the 50 veligers distributed in the controls died. Therefore, all dead experimental animals were attributed to predation.

DISCUSSION

Although often ignored in benthic community studies, predatory infauna are known to be an important component in regulating the densities and structure of such communities and may influence recruitment to the benthos by consuming settling larvae and early-stage juveniles (Woodin 1976, Commito 1982, Commito and Ambrose 1985 and citations therein). However, because predator-prey interactions are more easily studied for hard-bottom communities than for soft-bottom ones, more information is available for rocky shores than for the soft-bottom benthos (Commito and Ambrose 1985).

Some predator types that were tested in this study, including brachyuran crabs, hermit crabs, spiny lobster, and fishes, are also suspected or known predators of 1+ year class queen conch (>50 mm SL) (Randall 1964, Iversen et al. 1986). This study, however, specifically identifies a host of new micropredators capable of killing the youngest of the 0+ year class (≤ 5 mm SL) and represents the first published data on the subject.

All dorvilleids, most glycerids, most lumbrinerids, a few nereids, all phyllodocids, all sigalionids, and some syllids are consid-

ered carnivores, and the possession of eversible pharynges with jaws for feeding is characteristic of most of these polychaete families (Fauchald and Jumars 1979). At least one species tested from each of these families in this study killed either competent veligers or newly settled conch. Polychaete worms have not yet been reported in the literature as conch predators.

Crustaceans proved to be significant predators on newly settled conch. Brachyuran crabs are well-known predators of gastropods, and gastropods suffer high mortality as a result of predation by crushing (Vermeij 1977, Vermeij 1978). Crabs crush prey that are relatively small, sometimes severing the spire, and peel those that are too big to crush (Shoup 1968, Zipser and Vermeij 1978, Bertness and Cunningham 1981, du Preez 1984) because their chelae slip over the apical whorls when attacking larger shells from the spire (Lawton and Hughes 1985). Lobsters are also known to peel conch shells (Randall 1964, Davis 1992). Xanthid crabs initially position a shell in their claws for crushing and reposition it for peeling if further shell examination indicates a size too large for crushing, whereas shells that are extremely large are immediately positioned for peeling (Bertness and Cunningham 1981). Given that xanthid crabs can be very abundant ($>200 \text{ m}^{-2}$) in conch nurseries (Stoner et al. unpubl.), and that a single xanthid can consume 20 small (<2 mm SL) conch in 7 h in the laboratory (Ray et al. unpubl.), this crab family has the potential to highly affect survivorship of newly settled conch.

The filefish *M. ciliatus* also proved to be a capable predator of newly settled conch. Stoner (1990) attributed most of the post-settlement mortality of newly settled colonial ascidians to fish, and Brown and DeVries (1985) concluded that predaceous fish can decimate populations of thin-shelled, freshwater snails, prevent

them from becoming established, and potentially influence species composition of snail communities. Molluscivorous fishes and brachyuran crabs in both fresh and salt water can strongly influence the population dynamics, shell morphology, and geographic distribution of their snail prey (Palmer 1979, Vermeij 1977, Brown and DeVries 1985).

When large motile predators including crabs and fishes were excluded from cages in a shallow subtidal sand community, the densities of all infaunal species increased as a result of larval recruitment (Virnstein 1977). In treatments exposed to predators, the deeper-living infaunal species and those capable of making a quick retreat into the sediment, including the bivalve *Mya arenaria*, were more protected from predators living in the water column than those infaunal species that lived closest to the surface. Furthermore, Virnstein (1977) concluded that predation by blue crabs controlled the density of the shallow sediment-dwelling bivalve *Mulinia lateralis*—when the crab density was highest, the bivalve density was lowest. Therefore, even when conch bury themselves just below the sediment surface, they are probably highly vulnerable and easily detected and attacked.

High instantaneous rates of natural mortality (maximum mean = 12) have been calculated for 1+ juvenile conch (50–100 mm SL) and largely attributed to predation (Stoner and Glazer in press). Given the abundance of some micropredators in conch nurseries and their laboratory consumption rates (Stoner et al.

unpubl., Ray et al. unpubl.), we predict even higher mortality for newly settled conch than that determined for 1+ juveniles. The smallest conch for which predator-induced mortality data are available in the wild were 11 mm SL (~60 days in postmetamorphic age) (Ray and Stoner 1995). When these conch were tethered in well-established conch nursery areas, they suffered 50–96% predation-induced mortality in 11 days.

Settling larvae and newly settled queen conch are clearly vulnerable to a host of micropredators, some of which are found in high densities within conch nursery areas. Although this study identifies many species capable of killing the earliest stages of conch in the laboratory, the effects of micropredators in the wild remain to be quantified. Clearly, these micropredators can significantly affect the number of settlers that survive their first few months of postsettlement life and must be considered in studies of queen conch population dynamics.

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EFFECTS OF STOCKING DENSITY AND SUBSTRATE PRESENCE ON GROWTH AND SURVIVAL OF JUVENILE SPOTTED BABYLON, *BABYLONIA AREOLATA* LINK 1807 (NEOGASTROPODA: BUCCINIDAE)

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ABSTRACT Effects of stocking density and substrate presence on the growth and survival of juvenile spotted babylon, *Babylonia areolata*, were assessed over a period of 180 days. Juveniles, with an average shell length of 15.0 ± 0.4 mm ($n = 25$), were held in $1.0 \times 1.0 \times 0.8$ m flow-through rearing tanks supplied with filtered (1 μ m pore size) aerated seawater at a rate of 5 L/min. Juveniles were divided into four stocking densities at 50, 100, 150, and 200 individuals/m² with sand substrate or no sand substrate in the rearing tank. Juveniles were fed to satiation twice daily with fresh carangid fish, *Selaroides leptolepis*. The results showed that absolute growth rates in shell length and body weight of juveniles reared with sand were significantly ($p < 0.05$) higher than those without sand, but no significant differences were found among the densities. Average length increment ranged from 3.3 to 3.8 and 2.1 to 2.7 mm/mo for sand and no sand substrate, respectively. Mortality during the experiment, at all densities, was negligible. Mean survival ranged from 92 to 100% and 83 to 95% for sand and no sand substrate, respectively. It may be concluded that growth, in both shell length and body weight, and survival of juvenile *B. areolata* are considerably affected by the presence of substrate, but not by stocking density (up to 200 individuals per m²).

KEY WORDS: *Babylonia areolata*, growth, survival, stocking density, substrate presence

INTRODUCTION

Spotted babylon, *Babylonia areolata*, commonly known as Hoy Wan in Thailand, is an important commercial marine gastropod in Thailand. It is abundant and widely inhabits littoral regions in the Gulf of Thailand, especially muddy sand areas not exceeding 5–10 m in depth (Panichasuk 1996). This species spawns year-round, with a maximum peak in March. Average spawning interval is 6.5 days/mo. Size and age at maturity are 40.0 mm and 1 y old, respectively (Singhagraiwan 1996). The life history of *B. areolata* is characterized by the presence of eggs contained in capsules laid on muddy sand substrates; embryos develop inside the capsules, emerging as planktonic veligers 7 days after the capsules are deposited. Larvae are competent to metamorphose within 18 days after hatching. The metamorphosed larvae are benthic and spend most of their time immobile and partially buried in the sand, although they are capable of movements when offered prey or confronted by a predator (Chaitanawisuti and Kritsanapuntu 1997). Growth of *B. areolata* under hatchery conditions is 2.98 mm/mo in shell length, with survival exceeding 90%. They can reach a minimum marketable size of 4.0–4.5 cm shell length within 8 mo after hatching. The marketable size of spotted babylon is 4.0–6.5 cm in shell length, and the price of whole body weight is now 10 SUS/kg (Chaitanawisuti and Kritsanapuntu 1997).

The babylonia fishery is primarily in eastern and southern Thailand. The species is harvested from natural beds by means of baited traps. However, catch per unit effort has recently declined in traditional areas, particularly of larger organisms. Decreasing natural stocks and increasing value have led to an increased attention in the culture of this species as a means of preventing overfishing and increasing supply (Panichasuk 1996). *B. areolata* is now a promising new candidate for aquaculture, but further research is required to develop an economically viable operation (Munprasit and Wudthisin 1988, Morton 1990, Ayyakannu 1994, Raghunathan et al. 1994, Shanmugaraj et al. 1994, Singhagraiwan 1996).

The aim of this study was to assess the effects of stocking densities and substrate presence on the growth and survival of juvenile *B. areolata*.

MATERIALS AND METHODS

Preparation of Animals

Broodstock spotted babylon, *B. areolata*, with a mean shell length of 5.6 ± 0.3 cm ($n = 25$) were held in $2.0 \times 1.0 \times .08$ m spawning tanks supplied with flow-through seawater (5 L/min). Salinity and temperature ranged from 26 to 29 ppt and 28 to 29°C, respectively. A 10-cm layer of fine sand was provided as substrate. They were fed to satiation twice daily with fresh carangid fish, *Selaroides leptolepis*. The animals were cultured for 10–15 days until natural laying eggs occurred.

After laying eggs, egg capsules were collected and rinsed with filtered (1 μ m pore size) seawater. They were then placed in plastic baskets of 0.5-cm mesh size and submerged in $1.5 \times 0.5 \times 0.3$ m hatching tanks containing filtered (1 μ m pore size) ambient aerated seawater. Water was replenished daily until hatching. After hatching, the veligers were collected with a 200- μ m nylon mesh sieve and rinsed three times with filtered (1 μ m pore size) ambient seawater. These veligers were transferred to $1.5 \times 0.5 \times 0.3$ m larval rearing tanks containing filtered (1 μ m pore size) ambient aerated seawater. The initial stocking density was 10,000 larvae per liter. Larvae were primarily fed twice daily with 2.0×10^5 cells/mL of a mixture of *Isochrysis galbana* and *Tetraselmis* spp. (1:1). Water was changed every 2 days, and the rearing tank was washed with 0.3 ppm sodium hyperchlorite for 10 min and rinsed two to three times with wellwater. Spotted babylon larvae were competent to metamorphose within 18 days after hatching at a mean ($n = 25$) shell length of 1.520 ± 0.4 μ m, at which time they started settling on the bottom of the larval rearing tanks with no particular substrate provided.

After settling, the juveniles were transferred into $1.5 \times 0.5 \times 0.5$ m juvenile nursery tanks supplied with flow-through seawater (5 L/min). A 3-cm layer of fine sand was provided as substrate. The initial stocking density was 100 juveniles/m² to minimize detrimental effects of crowding on growth and survival. The food was changed from unicellular microalgae to chopped carangid fish, *S. leptolepis*, fed to satiation twice daily. Juveniles were cultured until the average shell length was 15 mm, which was used for the experiment.

Nursery System Designs

The experiment was conducted at the hatchery of Sichang Marine Science Research and Training Station, Chulalongkorn University, located on Sichang Island, the inner part of the Eastern Gulf of Thailand. Juvenile *B. areolata* were reared in 100-L fiberglass tanks with 1.0 m² total bottom area. The culture system was supplied with flow-through filtered (1 μ m pore size), aerated seawater at a rate of 5 L/min. Salinity and temperature ranged from 26 to 29 ppt and 28 to 29°C, respectively. Two types of substrates (sand substrate and no sand substrate) were compared in triplicate. The bottom of rearing tanks was covered with a 10-cm layer of fine sand as substrate, and the substrate was cleaned with a water jet and sun dried at 30-day intervals. The second was without sand substrate.

Stocking Density Experiment

Juveniles with an average shell length of 15.0 ± 0.4 mm ($n = 50$) were divided into four stocking densities of 50, 100, 150, and 200 individuals/m². They were then transferred to rear in the experimental nursery systems, as described below, over a 180-day period. The animals were fed chopped carangid fish, *S. leptolepis*, to satiation in the morning and evening. Size grading was not done for all treatments throughout the growout period. Shell length, shell width, and body weight were measured individually every month.

Data Analysis

On the basis of length and weight data obtained from the experiments, the absolute growth rates in shell length (G) were calculated from the average monthly increments in shell size according to the formula: $G = (L_1 - L_0)/(t_1 - t_0)$, where L_1 = length at time t_1 and L_0 = length at time t_0 (Wolff and Garido 1991). Final individual weight gain (W) and length increment (L) were calculated from the differences in mean body weight and shell length between the beginning and the end of the experiment. Total biomass gain (B) was the overall weight of each treatment at the end of the experiment. The number of dead individuals was recorded at monthly intervals, and an average monthly survival rate was calculated for all treatments.

All statistical analyses were performed with the SPSS/PC+ Statistical Package for the Social Sciences. Data on growth rate were subjected to log-transformation and survival rate was initially arcsine transformed before statistical analysis. Differences in growth and survival of spotted babylon among density groups and nursery systems were determined by two-way analysis of variance (ANOVA) (fixed factors: density and nursery system) at $\alpha = 0.05$. Tukey's studentized range tests ($\alpha = 0.05$) were used to compare between pairs of means if the ANOVAs were significant ($p < 0.05$).

RESULTS

Growth

The mean absolute growth rates in shell length of *B. areolata* among four stocking densities for both substrates over the entire period of the experiment are presented in Figure 1. A two-factor ANOVA showed that absolute growth rates of the sand substrate were significantly higher than those of no sand substrate ($p = 0.001$), but there was no significant difference among the four density groups for both substrates ($p = 0.08$). The average length increment of the sand substrate were 3.86, 3.61, 3.49, and 3.37 mm/mo at stocking densities of 50, 100, 150, and 200 individuals/m², respectively, and those of no sand substrate were 2.71, 2.62, 2.07, and 2.15 mm/mo, respectively (Fig. 2). At the end of the experiment, the final individual shell length increment of the sand substrate was significantly higher than those of no sand substrate ($p = 0.001$), but there were no significant differences among the four densities for both substrates ($p = 0.14$).

Total Biomass Gain

Total biomass gains of *B. areolata* among four stocking densities for both substrates at the end of the experiment are presented in Figure 3. ANOVA indicated that total biomass of animals stocked at low density was significantly lower than that at high density ($p = 0.01$) for both substrates. Total biomass of the sand substrate was significantly higher than that of no sand substrate (p

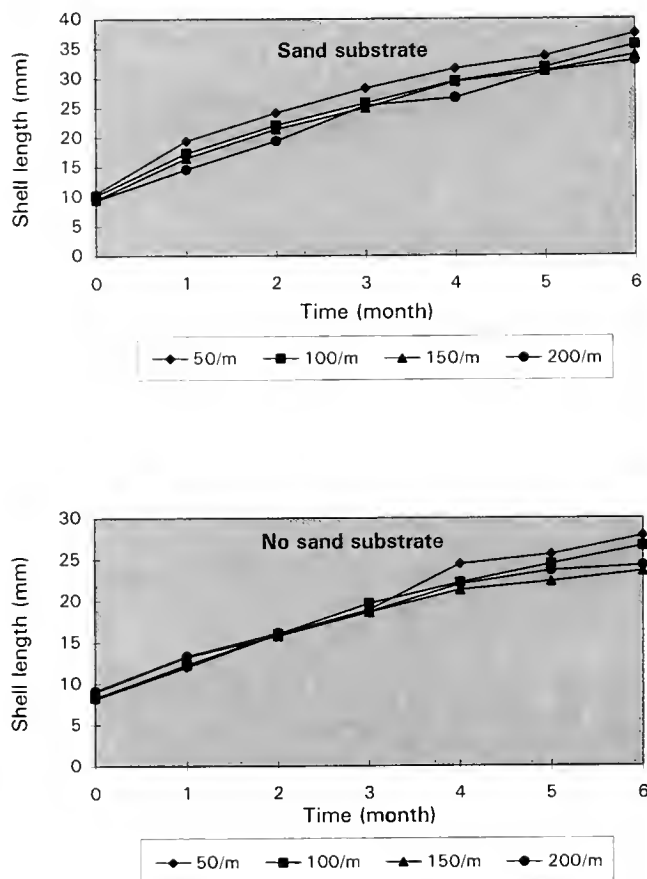


Figure 1. Average monthly growth in shell length of juvenile *B. areolata* at four stocking densities for both substrates.

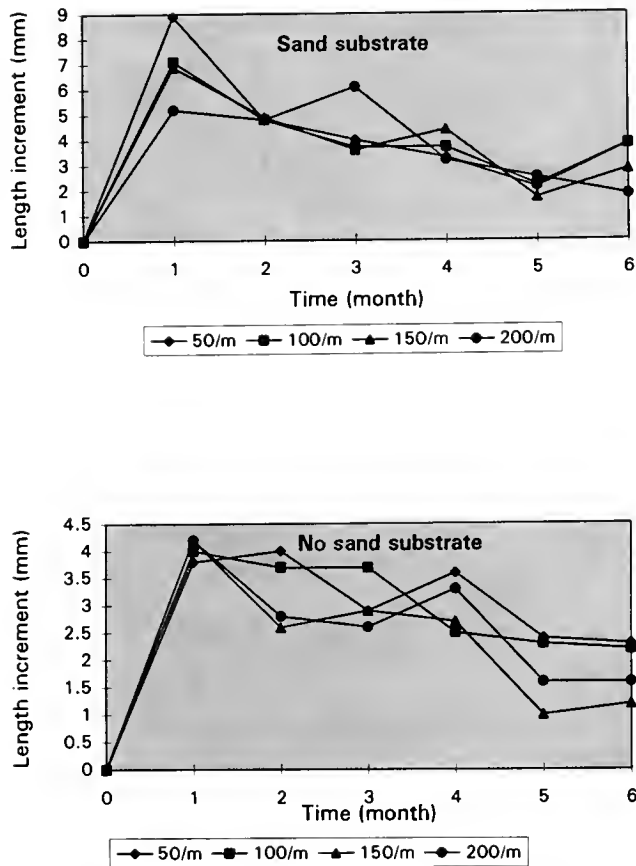


Figure 2. Monthly shell length increment of juvenile *B. areolata* at four stocking densities for both substrates.

= 0.03). An increasing trend in biomass gain as density increased was found for both substrates.

Survival Rate

Monthly survival rates of *B. areolata* at all densities for both substrates are presented in Figure 4. Mortality during the experiment at all densities was negligible. ANOVA indicated that survival of animals stocked at low density was not significantly higher than that at high density ($p = 0.42$) for both substrates. Survival rate of the sand substrate seemed to be higher than that of no sand substrate but was not significant ($p = 0.09$). At the end of the experiment, the range of the overall mean survival for all stocking densities was 92–100 and 83–95% for the sand substrate and no sand substrate, respectively.

Size Distribution

Size distributions of *B. areolata* among four stocking densities for both substrates at the end of the experiment are presented in Figure 5. At lower density, there was a higher proportion of the largest size class. Proportions of the largest size class (41–45 mm) were 25.0, 16.2, 1.7, and 3.3% for densities of 50, 100, 150, and 200 individuals/m² for the sand substrate, respectively, and those for no sand substrate were 10.5, 0, 6.7, and 1.2%, respectively.

DISCUSSION

The effect of stocking density and substrate presence on the growth and survival of *B. areolata*, examined under controlled

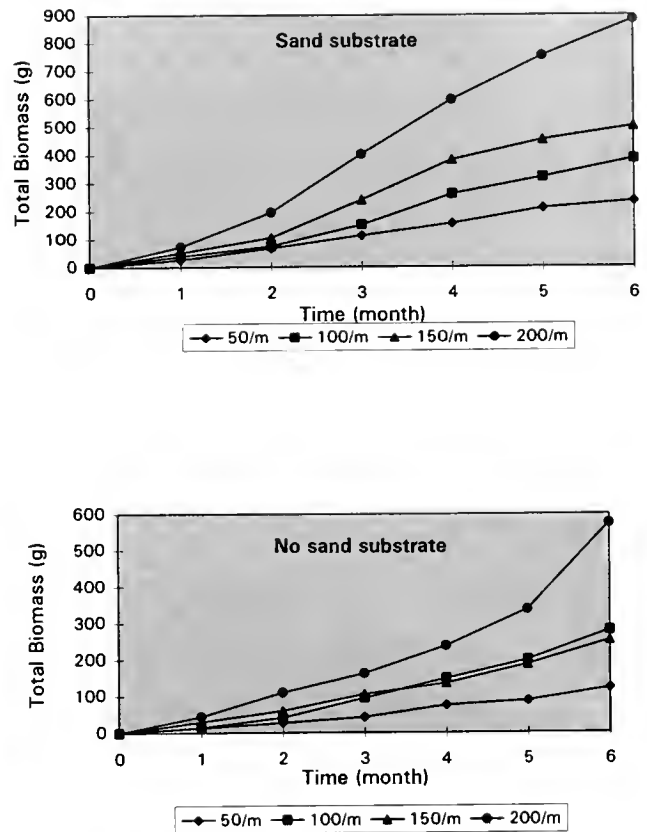


Figure 3. Monthly biomass gain of juvenile *B. areolata* at four stocking densities for both substrates.

conditions, was determined. This study revealed that for all stocking densities examined, the sand substrate resulted in better growth than no sand substrate (i.e., growth rate, shell length, and body weight were not influenced by density), but high survival was obtained at all densities. Chaitanawisuti and Kridsanapuntu (1997) reported that the maximum growth increment of *B. areolata* reared in a flow-through system or suspended culture, at stocking density of 100 individuals/m², was 2.98 and 2.01 mm/mo in shell length, respectively. Singhagrawan (1996) reported that the average growth increment of *B. areolata* with an average shell length of 5–6 mm, reared in a flow-through system at a stocking density of 100 individuals/m², was 3.14 mm/mo in shell length and 1.03 g in body weight. Many authors agreed with the relationship between growth and stocking density in shellfish culture. An inverse growth-density relationship existed and in general was caused by the interference between space and food competition (Hadley and Manzi 1984, Widman and Rhodes 1991, Allan and Maguire 1992, Hunt et al. 1995). *B. areolata* stocked at high densities seemed to implicate only space as a limiting factor but not food competition. Space was the main factor that restricted enlargement and was caused mostly by animals that survived and were buried under sand substrate at the bottom of the rearing tank. For food competition, high densities did not restrict the movement of animals in their search for food because various sizes of animals became clumped to the food and extended their proboscis to suck their food. It can be concluded that space limitation was probably the main factor affecting the growth of *B. areolata* at high densities because of shell enlargement. In this study, *B. areolata* showed higher

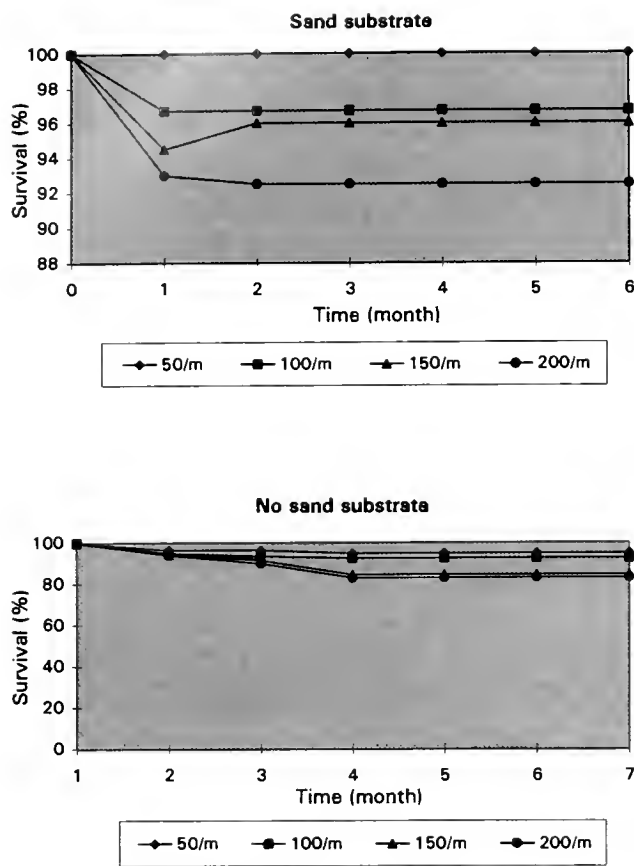


Figure 4. Monthly survival rate of juvenile *B. areolata* after harvesting at four stocking densities for both substrates.

growth rates than those of *Babylonia japonica* and *Babylonia spirata*. Doi (1975) reported that growth rates in shell length of juvenile *B. japonica* at 1, 3, and 5 y old were 4.2, 1.1, and 0.27 mm/mo, respectively. Nishihiro et al. (1985) report that growth of juvenile *B. japonica*, with average shell length of 18 mm, tagged and released into the sea, was 25 mm/y. Nishihiro et al. (1988) reported that 3 y after releasing juvenile *B. japonica*, with average shell length of 18 mm into the sea, the annual growth ranged from 35 to 57 mm with an average of 47.6 mm. Raghunathan et al. (1994) report that the average growth rate of *B. spirata* fed with clam *Meretrix meretrix* was 0.43 mm/mo under hatchery conditions. In addition, *B. areolata* showed higher growth rates than those of other commercially marine gastropods (giant muricid, trochus, and abalone). Nugranad et al. (1994) reported that juvenile *Chicoreus ramosus* reared in concret raceways could obtain a maximum shell increment of 97.24 mm over a period of 12 mo, with an average growth increment of 6.1 mm/mo. Fleming (1995) reported that the growth rate of the Australian abalone *Haliotis rubra* fed on red algae *Jeannerettia lobata* and *Laurencia botryoides* was 51.0 mg/day. Gimin and Lee (1997) reported that the growth rate of juvenile *Trochus niloticus* using fiberglass plates as

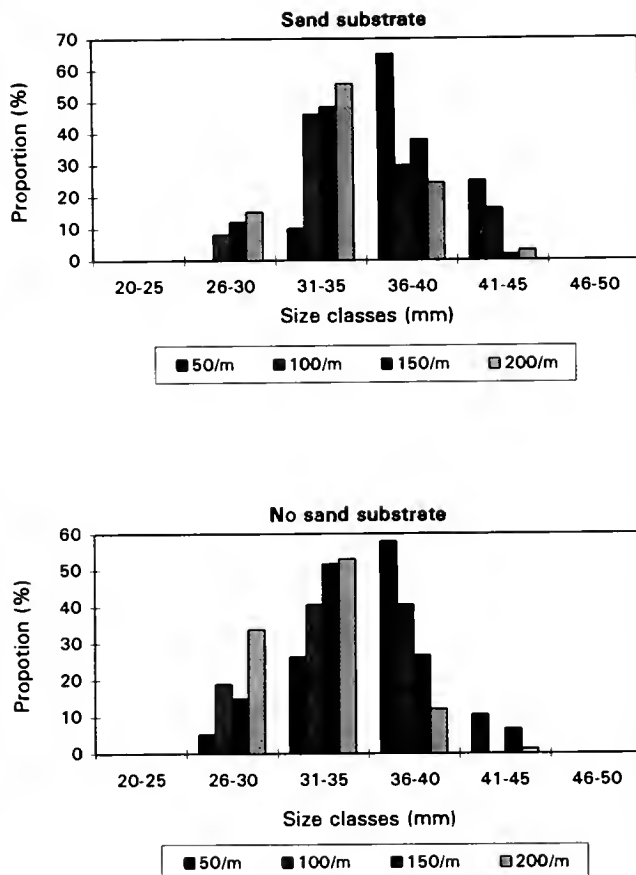


Figure 5. Size distribution of juvenile *B. areolata* after harvesting at four stocking densities for both substrates.

substrate was 101.6 $\mu\text{m}/\text{day}$. Consequently, any application of these results for commercial operation should be essentially preceded by size grading and thinning out of the animals at different growout periods to obtain maximum individual growth, survival, biomass gain, and economic considerations that may dictate densities that would result in a net reduction in overall production costs. However, the economic considerations play the most important roles in the success of this shellfish culture. For large-scale production, this species is recommended for culture at densities of as many as 200 individuals/ m^2 or more in a flow-through system with sand substrate.

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EVALUATION OF A GLUCOSE OXIDASE/PEROXIDASE METHOD FOR INDIRECT MEASUREMENT OF GLYCOGEN CONTENT IN MARINE MUSSELS (*MYTILUS EDULIS*)

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ABSTRACT A colorimetric method (glucose oxidase/oxidase) for indirect measurement of glycogen concentrations in tissue homogenates of marine mussels (*Mytilus edulis*) was evaluated. This method uses a conversion of glycogen to glucose by amyloglucosidase. Varying the buffer pH (4.5, 5.0, 5.5) and the amyloglucosidase concentration (160, 80, 40, 20, 10, 5, 1, and 0.5 mg/mL) did not appreciably optimize glycogen concentration. Coefficients of variation ($n = 10$) for mussel homogenates with mean glycogen concentrations of 94 and 334 mg/dL had within-run values of 0.75 and 0.96%, respectively. The between-run coefficients of variation ($n = 10$) for the same homogenates were 2.10 and 1.10%, respectively. When mean glycogen concentrations of thawed mussel homogenates were compared with those of initial fresh homogenates, a significantly ($p \leq 0.05$) lower glycogen concentration was seen in samples thawed after 1 day, but not in samples thawed after 1 h, 1 wk, or 1 mo. Glycogen recovery percentages of 99.3, 99.0, and 95.6% were obtained with mixed solutions containing 103.8, 95.2, and 10.8 mg/dL glycogen, respectively. The lower limit of sensitivity for the procedure was approximately 10 mg/dL. Because dilutions of a mussel homogenate with a high glycogen concentration (413.1 mg/dL) gave observed results within 5% of expected results, the assay was considered to be linear to at least 413.1 mg/dL. Glycogen concentrations based on analysis of wet tissue and lyophilized samples from 20 mature mussels were compared, resulting in a significant ($p \leq 0.05$) correlation coefficient of 0.52. An initial laboratory range (43–91 mg/g) for tissue glycogen based on wet weights (3.9–12.4 g) was determined with 20 mature mussels during July from the Morell region, Prince Edward Island, Canada. It was concluded that the colorimetric assay offered a reliable indication of tissue concentrations of glycogen in marine mussels (*M. edulis*).

KEY WORDS: glycogen, marine mussels, *Mytilus edulis*, method validation, spectrophotometric analysis

INTRODUCTION

Glycogen has been reported by Gabbott (1976) to be the primary carbohydrate used for energy in marine mussels (*Mytilus edulis*). Glycogen content of mussel tissues varies with the annual reproductive cycle, with the lowest concentrations occurring in midwinter at the time of gametogenesis (De Zwaan and Zandee 1972, Bayne 1973). The glycogen content of mussel tissues could influence disease resistance and commercial shelf life. To proceed with investigations of tissue glycogen in *M. edulis*, it is necessary to validate an analytic method for measuring glycogen in this species. Validation procedures such as assessing precision, recovery, linearity, and stability determine how reliable an assay is for the species of interest (Peters and Westgard 1986). Validation is mandatory before the use of any assay in a clinical chemistry laboratory for parameter measurement in human or veterinary patients (Peters and Westgard 1986, Murray et al. 1993). It appears logical, therefore, to continue with validation procedures for laboratory investigations in shellfish. The method reported by Carr and Neff (1984) and used most frequently to measure glycogen in shellfish tissues is based on the enzymatic conversion of glycogen to glucose via amyloglucosidase, followed by the determination of glucose concentrations by a commercially available oxidase/oxidase method. The purpose of the study reported here was to determine if a commercial assay for measuring glucose in human sera could be validated for indirect measurement of glycogen in tissue homogenates of marine mussels (*M. edulis*).

MATERIALS AND METHODS

Assay Procedure

A colorimetric glucose oxidase/oxidase method for indirect measurement of tissue glycogen (Carr and Neff 1984) was adapted for use in marine mussels. Mature (4–6 cm shell length) marine mussels (*M. edulis*) were obtained from a mussel lease in Prince Edward Island, Canada. They were removed from their shells, blotted dry, and weighed. They were individually homogenized in a small blender with 25 mL of buffer (0.1 M trisodium citrate, pH 5.0) for 30 sec. At all times in this protocol, the trisodium citrate buffer was ice cold at the time of addition to the mussel tissue. The mixture was then transferred to a 250-mL Erlenmeyer flask, and a further 75 mL of buffer was used to rinse the small blender. These washings were added to the flask. The blend was heated in a boiling water bath for 5 min. After cooling to room temperature, the mixture was rehomogenized for approximately 1 min in a larger blender until a fine slurry was obtained. A 5.0-mL aliquot of the homogenate was incubated with 500 μ L of a 0.5% solution in 0.1 M trisodium citrate buffer (pH 5.0, sterile filtered, 104 U/mL) of amyloglucosidase (from rhizopus mold; Sigma Chemical Co., St. Louis, MO). Another 5.0-mL aliquot was incubated with the same volume of buffer (but without amyloglucosidase) to serve as a blank. *M. edulis* glycogen (Type VII from mussel; Sigma Chemical Co.) standards (100 and 200 mg/dL) were prepared in 0.1 M trisodium citrate buffer (pH 5.0) and were treated identically to the

tissue samples. Aliquots containing amyloglucosidase and those without amyloglucosidase (blanks) were incubated at room temperature (20–24°C) for 16 h. After incubation, the samples were centrifuged at 1,300 g for 30 min. The glucose content of the supernatants (enzyme treated and enzyme untreated) was determined using benchtop techniques and a commercial reagent for glucose (Glucose Trinder reagent; Diagnostic Chemicals Ltd., Charlottetown, Prince Edward Island, Canada). Glycogen standards were analyzed with each run, and glycogen concentrations were expressed as: $G_m = [(T-U-A)_m \times S \times V] / [(T-U-A)_s \times W]$, where: G_m = glycogen concentration expressed as mg/g of mussel tissue, $(T-U-A)_m$ = absorbance reading of enzyme-treated aliquot (T)—absorbance reading of enzyme-untreated aliquot (U)—absorbance contribution due to amyloglucosidase (A) for each sample, S = concentration of glycogen standard (mg/dL), V = volume of extracting solution in dL, $(T-U-A)_s$ = same designations as for T , U , and A as above, but for standard samples, and W = weight of tissue sample in grams. The correction factor ($-A$) is required in this formula, because commercially available amyloglucosidase contains a small amount of glucose. Glycogen concentrations were alternatively expressed as: $G_c = [(T-U-A)_m \times S] / [(T-U-A)_s]$ where G_c = glycogen in mg/dL. This latter form was used for precision and recovery studies where solutions containing known glycogen concentrations were desired.

Assay Optimization

Mussel weight to buffer volume was optimized at 1 mussel to 100 mL, because earlier attempts to use less buffer resulted in glucose concentrations that were beyond the upper limit of linearity of the assay. To determine if the conversion of mussel glycogen to glucose could be further optimized, final citrate buffer solutions with pH values of 4.5, 5.0, and 5.5 were evaluated. To achieve this, two mussels were homogenized separately for 30 sec, each in 20 mL of 0.1 M trisodium citrate buffer (pH 5.0). After a 5-min incubation in a boiling water bath and cooling to room temperature, the mixtures were rehomogenized to a fine slurry (1 min) and divided into three aliquots of 5.0 mL each. To these aliquots, 20 mL of 0.1 M sodium citrate buffer of varying pH levels was added to obtain final buffer pH values of 4.5, 5.0, and 5.5. The different aliquots were then treated as above, with preparations of enzyme-treated and enzyme-untreated samples prepared. To determine if the concentration of amyloglucosidase could be optimized for maximal conversion of mussel glycogen to glucose, amyloglucosidase solutions with concentrations of 160, 80, 40, 20, 10, 5, 1, and 0.5 mg/mL were prepared in 0.1 M trisodium citrate buffer (pH 5.0). After the rehomogenization, 5.0-mL aliquots of homogenate from separate mussel samples were each incubated with the amyloglucosidase solutions described above. Enzyme-untreated (blank) solutions and standards were prepared as described previously.

Assay Evaluation

To evaluate the precision of the assay, within-run and between-run (day-to-day) studies were conducted and coefficient of variation (CV) calculations were performed. Two samples of mussel homogenate (post-boiling water bath and rehomogenization) with mean glycogen concentrations of 94 and 334 mg/dL were analyzed 10 times to obtain data for the within-run calculations. Aliquots of

the same homogenates were frozen, thawed, and analyzed 10 times on separate runs over a period of 3 wk to obtain the between-run precision. In order to determine frozen stability of glycogen samples, an additional 10 individual mussel samples were frozen at -29°C in separate aliquots after heating and rehomogenization, as described previously. These were thawed after periods of 1 h, 1 day, 1 wk, and 1 mo (4 wk), and the glycogen concentrations were determined. Assay recovery capabilities were assessed by determining the glucose concentrations of mixtures containing homogenates of known glycogen concentrations (235 and 153 mg/dL) combined with the 90 mg/dL glucose standard provided in the commercial kit in a 1:9 proportion by volume.

The minimum amount of converted glycogen that could be reliably measured by the assay was evaluated. To accomplish this, mixtures (1:1, 1:3, and 1:7 by volume) formed by combining two homogenates containing 22.5 and 215 mg/dL glycogen, respectively, were prepared. Baseline mixtures containing buffer and homogenate from the 215 mg/dL blend were prepared in the same ratios. Both sets of mixtures were analyzed, and the amount of glycogen recovered was determined. The linearity of the assay was evaluated by measuring the glycogen concentrations of a set of serial dilutions (in 0.1 M trisodium citrate buffer [pH 5.0]) of a mussel homogenate sample with a high glycogen concentration of 413.1 mg/dL. The dilutions, based on percentages of the previous sample in the series (with expected results in brackets expressed as mg/dL), were as follows: 100% (413.1), 75% (309.8), 66.7% (206.6), 50% (103.3), 50% (51.6), and 50% (25.8). To evaluate the possible influence of variable tissue water content on the expression of glycogen concentration, 20 mature mussels were processed as described previously and divided into aliquots after the final rehomogenization step. For each of the 20 samples, one aliquot (11 mL) was frozen at -29°C . After thawing, aliquots of this material were analyzed for glycogen content as described previously, with the glycogen concentration expressed as mg/g wet tissue. A second aliquot (20 mL) was dispensed into a 50-mL serum bottle (Wheaton "400" borosilicate glass; Wheaton, Millville, NJ), lyophilized in a freeze dryer (Labconco Corporation, Kansas City, MO), and stored in a refrigerator (4°C). The lyophilized sample was weighed (weight corrected for buffer salt content), dissolved in 20 mL of 0.1 M trisodium citrate buffer (pH 5.0), and analyzed for glycogen content. The glycogen concentrations were expressed as mg/g dry tissue.

Statistical Analysis

A computer software program (Minitab Statistical Software Inc., Version 9.1, State College, PA) was used for statistical calculations. All tests were performed at the $p \leq 0.05$ significance level. A repeated-measures analysis of variance calculation was performed to determine if significant differences existed between mean glycogen concentrations of fresh and frozen aliquots of the same tissue homogenate. Linear regression analysis was performed for the comparison between glycogen concentrations expressed as wet weights and dry weights for the 20 samples in which tissue homogenates and lyophilized samples were available. An initial laboratory range for tissue glycogen content using the described method was determined by using the lowest to highest values for 20 mature (4–6 cm shell length) mussels in July from the Morell region, Prince Edward Island, Canada.

RESULTS

Varying the buffer pH and changing the concentration of amyloglucosidase in the reagent mixture resulted in no appreciable optimization in glycogen concentrations in mussel samples (Table 1). Therefore, the buffer pH of 5.0 and an amyloglucosidase concentration of 0.5% previously reported (Carr and Neff 1984) were used throughout this investigation.

The results of the precision study are summarized in Table 2. Coefficients of variation for within-run and between-run analyses were less than 3% in all calculations. When mean glycogen concentrations of thawed aliquots of mussel homogenates were compared with those of fresh aliquots, a significant ($p \leq 0.05$) difference was observed between samples thawed after 1 day (Table 3). However, no significant ($p \leq 0.05$) differences were observed between fresh aliquots and samples thawed after 1 h, 1 wk, or 1 mo. Recovery percentages obtained with solutions containing 10.8, 95.2, and 103.8 mg/dL glycogen ranged from 95.6 to 99.3% (Table 4). However, solutions containing lower than 10.8 mg/dL glycogen had unacceptably high recovery percentages. Because dilutions of a mussel homogenate with a high glycogen concentration (413.1 mg/dL) gave observed results within 5% of expected (Fig. 1), the colorimetric assay was considered linear to at least 413.1 mg/dL. The relationship between glycogen concentrations of 20 samples expressed as wet and dry weights is shown in Figure 2; the significant correlation coefficient (r) was 0.52. Glycogen concentrations for a group of 20 mature mussels at a specific time (July) and location (Morell region, Prince Edward Island, Canada) ranged from 43 to 91 mg/g of wet tissue. The wet tissue weight varied from 3.9 to 12.4 g.

DISCUSSION

The colorimetric assay evaluated in this study was determined to be a reliable indirect indicator of tissue glycogen concentrations in marine mussels (*M. edulis*). The precision of the assay is acceptable, with coefficients of variation of less than 1% and less than 3% for the within-run and between-run evaluations, respectively.

Linearity as determined by our evaluation (413.1 mg/dL) was

TABLE 1.

Optimization data using different pH levels and amyloglucosidase concentrations for a colorimetric method for indirect glycogen measurement in marine mussels (*M. edulis*).

Parameter	Sample 1 Glycogen (mg/dL)	Sample 2 Glycogen (mg/dL)	Sample 1 Glycogen (mg/dL)	Sample 2 Glycogen (mg/dL)
pH				
4.5	33.7	25.6		
5.0	33.0	26.4		
5.5	33.7	25.3		
Amyloglucosidase (mg/mL)				
160			28.3	22.6
80			28.3	24.0
40			27.8	24.7
20			28.7	23.4
10			29.6	23.8
5			28.5	23.8
1			28.2	24.6
0.5			29.0	25.2

TABLE 2.

Precision data for a colorimetric method for indirect glycogen measurement in marine mussels (*M. edulis*).

Glycogen Concentration Range	Low	High
Within-run		
Mean glycogen concentration (mg/dL)	94	334
n	10	10
Standard deviation	0.71	3.22
Coefficient of variation (%)	0.75	0.96
Between-run		
Mean glycogen concentration (mg/dL)	92	329
n	10	10
Standard deviation	1.93	3.61
Coefficient of variation (%)	2.10	1.10

judged to be good, because serial dilutions of this high-concentration sample resulted in values within 5% of expected concentrations (Fig. 1). Linearity of the extraction procedure is limited to the linearity of the commercial reagent system used.

Initial steps in using this procedure were to evaluate if the conversion of mussel glycogen to glucose could be optimized by changing the buffer pH and/or the amyloglucosidase concentrations to those other than the previously published values of pH 5.0 and 0.5% amyloglucosidase (Carr and Neff 1984). This amyloglucosidase concentration can also be expressed in terms of activity (104 U/mL, using specific commercial reagents as described earlier). Although sample numbers in this analysis were limited, glycogen concentrations achieved using different pH levels and different amyloglucosidase concentrations showed no optimization and varied by less than 8%. Therefore, previously published values for pH and amyloglucosidase concentrations were used for all runs.

Correlation results of the glycogen concentrations of mussels expressed in terms of wet weight and in terms of dry weight were acceptable, although the significant ($p \leq 0.05$) correlation coefficient was not high (0.52). This is likely because of the variable

TABLE 3.

Stability data for a colorimetric method for indirect glycogen measurement in marine mussels (*M. edulis*).

Sample No.	Glycogen (mg/g)				
	Fresh	Frozen 1 h	Frozen 1 day	Frozen 1 wk	Frozen 1 mo
1	52.9	53.4	51.8	52.9	51.6
2	38.6	37.8	36.8	38.2	37.6
3	43.3	42.7	42.6	42.9	43.3
4	42.8	44.1	43.1	43.6	43.8
5	38.0	38.7	37.9	38.3	38.2
6	31.9	33.1	31.4	31.7	31.6
7	47.6	48.2	46.5	46.6	48.2
8	35.2	36.0	35.2	35.2	36.0
9	29.5	29.7	28.7	29.5	29.9
10	44.2	44.0	43.4	44.4	45.1
Mean	40.4	40.8	39.7	40.3	40.5
Mean % difference from fresh		1.0	1.6	0.2	0.3

TABLE 4.

Recovery data for a colorimetric method for indirect glycogen measurement in marine mussels (*M. edulis*).

Sample Description	Expected Glycogen (mg/dL)	Observed Glycogen (mg/dL)	Recovery Percentages*
Mixture 1	104.5	103.8	99.3
Mixture 2	96.2	95.2	99.0
Mixture 3	11.3	10.8	95.6
Mixture 4	5.6	7.4	132
Mixture 5	2.8	3.9	134

A, mussel tissue homogenate (235 mg/dL); B, commercial glucose standard (90 mg/dL); C, mussel tissue homogenate (153 mg/dL); D, mussel tissue homogenate (22.5 mg/dL); E, mussel tissue homogenate (215 mg/dL); F, 0.1 M trisodium citrate buffer (pH 5.0). Mixture 1 = (1 volume A + 9 volumes B); Mixture 2 = (1 volume C + 9 volumes B); Mixture 3 = (1 volume D + 1 volume E) - (1 volume F + 1 volume E); Mixture 4 = (1 volume D + 3 volumes E) - (1 volume F + 3 volume E); Mixture 5 = (1 volume D + 7 volumes E) - (1 volume F + 7 volumes E).

* Recovery percentages = (observed concentration)/(expected concentration) \times 100.

water content in the wet weight samples. If an absolute value for glycogen concentration is required, the dry weight analysis is the method of choice. However, if a less accurate method is acceptable, the wet weight method might be used. This latter method could be useful to commercial growers desiring quick results in order to predict shelf life. Wet weight glycogen analysis can be performed in a short time (24 h or less) compared with the 4–5 days required for the dry weight determination. In the study reported here, it was convenient to process samples at room temperature over a 24-h period. However, incubation of samples in a water bath at 55°C for 2 h is also reported (Carr and Neff 1984) and would speed analysis.

Acceptable recovery percentages (defined as approximately 90–110%) were obtained with glycogen samples at high values of 103.8 and 95.2 mg/dL and at values as low as 10.8 mg/dL. Solutions with glycogen concentrations lower than this (7.4 and 3.9

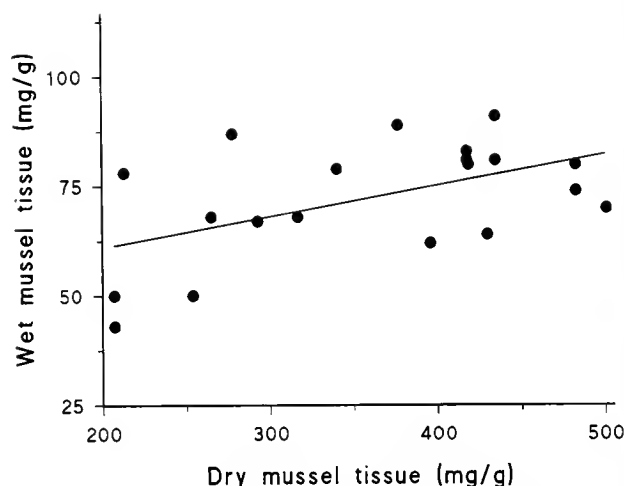


Figure 2. Regression analysis plot comparing glycogen concentrations of 20 marine mussels (*M. edulis*) expressed as both wet weights (mg/dL) and dry weights (mg/g). The regression equation is "wet = 46.6 + 0.712 dry," and the significant ($p \leq 0.05$) correlation coefficient r was 0.52.

mg/dL) demonstrated unacceptably high recovery percentages. Therefore, the value of 10 mg/dL is a good approximation of the lower limit of sensitivity for the assay. In the linearity assessment performed in this study, the lowest evaluated glycogen concentration was approximately 25.8 mg/dL. This sample performed well in the linearity assessment (i.e., was within 5% of expected value), but lower levels were not assessed.

Frozen tissue homogenates of marine mussels were determined to be stable for at least 1 month. Samples frozen for 1 hour, 1 week and 1 month displayed no significant difference in mean glycogen concentrations compared with the mean glycogen concentration of the fresh homogenates. Samples thawed after 1 day exhibited a statistically significant decrease in mean glycogen concentration as compared with fresh tissue. Because no significant difference was seen in the mean glycogen concentrations after freezing for longer times, it is unlikely that the slight decrease in mean glycogen concentration at the 1-day period was due to sample deterioration. Instead, between-run variability, due to subtle changes in pipetting technique or laboratory temperature, likely accounts for this change. Although there are insufficient data on what constitutes a clinically significant change in tissue glycogen of marine mussels, this decrease at 1 day represents a drop of only 1.6% compared with the fresh homogenates.

It is important to note that all glycogen concentrations achieved in this study were based on values determined after heating of the tissue homogenates. Heating inactivates endogenous glycogenases in mussel tissue, which could alter the glycogen concentration obtained using the enzymatic glucose analysis (Carr and Neff 1984). This step of heating the samples in a boiling water bath is therefore a tedious, but necessary, step. Easier methods of heating samples, such as microwave use, may be evaluated in future work.

In the analysis of 20 mature mussels for initial laboratory range determination, glycogen concentrations showed a wide range (43–91 mg/g). Because the number of individual animals assessed (20) was minimal, this should not be considered a proper reference range. Ideally, a minimum of 40–50 normal individuals is used for determination of a reference range (Lumsden and Jacobs 1989). Shellfish biochemical data may show high variability (Ruiz et al.

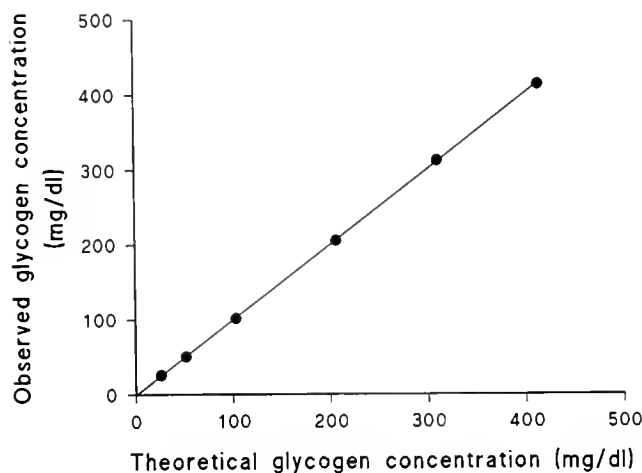


Figure 1. Linearity plot of a tissue homogenate of a marine mussel (*M. edulis*) with a high glycogen concentration (413.1 mg/dL) diluted in buffer (0.1 M trisodium citrate [pH 5.0]). Observed glycogen concentrations correlate closely (within 5%) with expected concentrations.

1992), making either a reference range based on numerous individuals or the use of pooled samples ideal. Also, any reference range in shellfish must be designated as specific for the time of year and geographic location. As previously noted, glycogen concentrations in marine mussels vary with reproductive activity. Therefore, more work is necessary to derive reliable reference ranges for whole-body glycogen concentration in marine mussels for each geographic region. Once accomplished, however, this information could be used for basic biologic experiments as well

as to determine if tissue glycogen concentrations affect resistance to disease and shelf life.

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LOW EFFECTIVE SIZES IN HATCHERY POPULATIONS OF THE EUROPEAN OYSTER (*Ostrea edulis*): IMPLICATIONS FOR THE MANAGEMENT OF GENETIC RESOURCES

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ABSTRACT Data on allozyme frequencies were used to estimate the effective sizes (N_e) of three hatchery-obtained populations of *Ostrea edulis* from Spain and France in the first hatchery generation. Two methods of N_e estimation were used: the so-called "temporal method," based on the changes of allele frequencies across generations, and the "heterozygosity method," based on the decrease of heterozygosity with respect to the parental wild population from which the broodstock animals were obtained. For comparison, the effective size of the wild progenitor population of one of the Spanish hatchery populations (Ortigueira) was also estimated by the temporal method. Large differences between the number of individuals used as broodstock and N_e were observed. More important, the estimates indicate that the N_e of hatchery populations is smaller than that of the wild population studied. Introduction in the wild of such low-variability hatchery-produced oysters could result in the reduction of inbreeding and variance N_e of the wild populations.

KEY WORDS: effective population size, supportive breeding, oyster, *Ostrea edulis*, allozymes

INTRODUCTION

The commercial exploitation of bivalves has been traditionally based on the collection of animals from natural beds, sometimes aided by simple husbandry techniques, such as the installation of collectors to increase settlement. In recent decades, the hatchery technology has allowed reproduction in captivity, in conditions that maximize the reproductive ability of the parents and larval survival. This facilitates the production of large amounts of "seed," which can be used for outgrow or restocking of natural populations.

From a genetic point of view, the use of a relatively small number of broodstock in a hatchery (certainly smaller than the number of reproducing individuals in the wild populations) has important consequences. The most important is that genetic drift is expected to be stronger than in wild populations, which will lead to a reduction of genetic variability, as well as an increase of the inbreeding rate of the population in successive generations. Furthermore, recent theoretical investigations have shown that the release of hatchery seed in wild populations for restocking may also have undesired consequences (loss of genetic diversity and inbreeding) for the population subject to restocking (Ryman 1991, Ryman 1994, Ryman and Laikre 1991, Ryman et al. 1995).

Polymorphisms in enzyme-coding genes (allozymes) have been used repeatedly to test the effects of genetic drift in hatcheries. Initial surveys of allozyme variability in hatchery populations did not show apparent effects of hatchery practices on the allozyme variability of those populations (Gosling 1982, Dillon and Manzi 1988, Wada 1986, Vrijenhoek et al. 1990). Hedgecock and Sly (1990) used a more sophisticated statistical methodology to address the question (see also Hedgecock et al. 1992). They estimated the genetically effective sizes (N_e) of hatchery populations of bivalves from variances in gene frequencies of allozyme markers across generations. They found that N_e in hatcheries is usually smaller than in the wild progenitor populations and is often much lower than the number of animals used as broodstock. Because N_e is a predictor of the strength of the genetic drift, their findings indicated that hatchery populations were usually affected by an important amount of drift.

The European oyster, *Ostrea edulis*, is exploited in large areas of the European coasts and has also been introduced to North

America. Natural populations have been overfished and affected by parasite epidemics in a large part of their range of distribution. Hatchery-obtained seed is very often used for outgrow and restocking of wild beds. Several authors have reported important changes in gene and genotype frequencies and losses of heterozygosity at allozyme loci in hatchery populations when compared with the wild oyster beds from which they were derived (Wilkins 1975, Alvarez et al. 1989, Saavedra and Guerra 1996). However, a systematic study of genetic drift in oyster hatcheries has not been attempted. The purpose of this article is to investigate the effective sizes of the hatchery populations of *O. edulis*. Because hatchery-produced oyster seed is widely used to replenish natural oyster beds, the consequences of such practice on natural populations of *O. edulis* will be also examined.

MATERIALS AND METHODS

Populations Studied

Three hatchery populations that were studied for allozyme frequencies by different authors, and for which information on allozyme frequencies in the wild populations from which they were derived was also available, will be considered in this study: Ortigueira (Spain), Ribadeo (Spain), and St. Vaas-le-Hougue (France). The first is a hatchery population obtained in 1983 from 60 parental oysters taken from the Ría de Ortigueira (northwest Spain). A sample of 419 18-mo-old individuals from this population was scored for five allozyme polymorphisms by Alvarez et al. (1989). Data for the wild population come from a sample of 97 individuals taken in 1985 and studied for allozyme polymorphisms by Saavedra et al. (1987) (their ORT-1 sample). The second hatchery population was derived from 120 breeders taken from the Ría de Ribadeo (northwest Spain) in 1988 and studied for allozymes by Saavedra and Guerra (1996) (sample size was $n = 1,212$). Allozyme data for the wild population come from a sample of 86 individuals taken at the same time that the breeders were taken (the RIB population in Saavedra et al. 1993). The number of polymorphic allozyme loci common to both hatchery and wild populations was nine. During the growout period, the hatchery population of Ribadeo was maintained as three independent replicates (Saavedra and Guerra 1996). Because replicates were es-

established after the foundation of the population, and no significant differences in gene frequencies were observed between replicates (Saavedra unpubl.), they have been pooled for this study. Finally, the hatchery population from St. Vaas-Hougue was obtained in 1980, and a sample of 50 individuals was studied for allozyme polymorphisms by Le Pennec et al. (1985). The authors do not give the number of parental individuals. A sample of 31 individuals taken from the wild population was studied by the same authors, and data are available for four polymorphic allozyme loci common to both samples. The two Spanish populations are representative of those used for growout or replenishment of wild oyster beds. The French population was also used for cultivation (Le Pennec et al. 1985).

To compare hatchery and wild effective population sizes in *O. edulis*, the estimation of N_e was also carried out in the wild population of Ortigueira. For this purpose, the ORT-1 sample studied by Saavedra et al. (1987), obtained in 1985, was compared with a sample from the same population obtained in 1989 and scored for allozyme polymorphisms following the techniques of Saavedra et al. (1993). Allozyme frequencies for the 1989 sample are given in Table 1. The estimated census size of this oyster population was 10,000 (A. Guerra, Centro de Investigaciones Mariñas, pers. comm.).

Estimation of N_e

The estimation of N_e from temporal changes of gene frequencies (hereafter referred to as the "temporal method") was done as described in Hedgecock et al. (1992). A review of the methodology is given by those authors and by Waples (1989). Briefly, it consists of the calculation of the standardized temporal variance of gene frequencies for each locus (F_k) in samples separated by t generations, following the methods of Nei and Tajima (1981) and Pollak (1983). The average of F_k across loci (F^*), weighted by the number of alleles, was then used to estimate the value of N_e by the expression

$$N_e^* = t / (2[F^* - 1/2S_0 - 1/2S_t])$$

where N_e^* represents the estimate of N_e , and S_0 and S_t are the harmonic averages of sample sizes across loci in generations 0 and t , respectively (Waples 1989). The time interval between generations in the above expressions was $t = 1$ in all hatchery populations. Demographic studies of wild oyster populations are not available, so an estimate of generation length can only be guessed. In the wild population of Ortigueira, oysters reach commercial size in the second summer after settlement, and then most of them are fished. Therefore, a generation time of 2 y can be assumed. On the basis of this estimate, an interval of $t = 2$ generations between 1985 and 1989 was considered for the estimation of N_e^* . Standard

errors for N_e^* were calculated by using a χ^2 approximation (see Waples 1989).

Because the number of loci scored in some of the populations studied here was low, the estimates are affected by a large error (Waples 1989). In order to contrast the estimates obtained through the temporal method, the N_e of hatchery populations was also estimated from the changes in expected heterozygosity. In a population of size N_e , the initial heterozygosity (H_0) will decrease to H_t after t generations. The relationship between H_0 and H_t is given by the equation $H_t = H_0 (1 - 1/2N_e)^t$ (Crow and Kimura 1970) and allows an estimate of N_e from the expected heterozygosities in the hatchery (H_t) and in the wild progenitor population (H_0). Here, H_t and H_0 were estimated by averaging single-locus unbiased heterozygosities, h_t and h_0 (Nei 1978). Confidence intervals for the heterozygosity estimate of N_e were obtained by jackknifing over loci.

An assumption of the methods used for estimating N_e is that the allozyme polymorphisms studied are neutral with respect to natural selection. In order to test this assumption, the methods of Hedgecock and Sly (1990) and Hedgecock et al. (1992) were used. One method takes advantage of the fact that, under neutrality, $nF/E(F)$ follows approximately a χ^2 distribution with n degrees of freedom, with n being the number of loci scored, and $E(F)$ being the expected value of the drift variance (Waples 1989). The distribution of nF_k/F^* can be compared with the expected values under a χ^2 (with n degrees of freedom [df]) in a probability plot and the goodness of fit tested by the Kolmogorov-Smirnov (K-S) test. A second test for neutrality is to compare the number of alleles from the wild population that remain in the hatchery population with that expected if N_e^* was the true N_e . Statistical significance is checked by a χ^2 test (see Hedgecock and Sly, 1990 for details).

Calculations of effective population sizes by the temporal method and the test of neutrality based on the number of alleles lost were performed with the program EPS, provided by D. Hedgecock and V. Chow (Bodega Marine Laboratory). Goodness of fit of the distribution of nF_k/F^* to the χ^2 distribution was done with SYSTAT.

RESULTS

Estimates of single-locus heterozygosities and standardized temporal variances of gene frequencies are presented in Table 2. The estimates of N_e for each population using both heterozygosity and temporal methods are given in Table 3.

Effective Size of the Wild Population

The estimate of N_e for the wild population of Ortigueira obtained from the temporal variance of gene frequencies was 248, although the upper limit of the 95% confidence interval (CI) in-

TABLE 1.
Allele frequencies at five allozyme loci in the 1989 sample from the wild population of Ortigueira.

Locus:	<i>Est-3</i> (n = 316)		<i>Idh-2</i> (n = 329)			<i>Mdh-1</i> (n = 314)		<i>Pgi</i> (n = 329)		<i>Pgm</i> (n = 302)			
Allele:	90	100	89	100	113	100	131	62	100	90	100	112	120
	0.065	0.935	0.046	0.953	0.002	0.804	0.196	0.030	0.970	0.013	0.512	0.469	0.007

* n, number of individuals sampled.

TABLE 2.

Number of alleles (n_a), single-locus heterozygosities in the wild source population (h_o) and in the hatchery populations (h_t), and single-locus estimates of the temporal variances of gene frequencies (F_k) in four populations of European oyster.

Locus	Ortigueira					Ribadeo				St. Vaas-la-Hougue			
	n_a	h_o	Wild F_k	Hatchery h_t	F_k	n_a	h_o	h_t	F_k	n_a	h_o	h_t	F_k
<i>Aldh</i>	—	—	—	—	—	4	0.176	0.035	0.0439	—	—	—	—
<i>Ark</i>	—	—	—	—	—	2	0.492	0.407	0.0978	—	—	—	—
<i>Est-3</i>	2	0.239	0.0589	0.185	0.0121	2	0.149	0.254	0.0449	—	—	—	—
<i>Est-5</i>	—	—	—	—	—	3	0.364	0.428	0.0277	—	—	—	—
<i>Idh-2</i>	2	0.058	0.0074	0.082	0.0048	2	0.132	0.199	0.0201	—	—	—	—
<i>Mdh-1</i>	3	0.289	0.0085	0.258	0.0071	2	0.262	0.151	0.0504	2	0.149	0.259	0.0496
<i>Me-1</i>	—	—	—	—	—	3	0.214	0.115	0.0242	—	—	—	—
<i>Pgdh</i>	—	—	—	—	—	—	—	—	—	2	0.200	0.000	0.2260
<i>Pgi</i>	2	0.043	0.0025	0.060	0.0031	2	0.079	0.069	0.0007	2	0.180	0.000	0.2000
<i>Pgm</i>	4	0.516	0.0008	0.508	0.0043	4	0.531	0.441	0.0343	2	0.557	0.420	0.2950

cluded infinity, and the lower limit was 30. This high uncertainty of the N_e estimate was probably the result of the small number of loci analyzed. Tests of neutrality were not significant. The number of actual remaining alleles was 12, whereas the expected number was 12.8 ($\chi^2 = 0.020$ p > 0.5). Observed values of $nF/E(F)$ were plotted against their expectation according to the χ^2 distribution for n degrees of freedom, and no significant departure from expected distribution was found. However, the fit of the distribution to a χ^2 with 4 df was poor (p = 0.062, by the K-S test). In this population, the locus *Est-3* showed a value of $F_k = 0.0589$ (Table 2), which was very high compared with those obtained for other loci (0.0008–0.0085), and resulted in a value of $4F_k/F^* = 18.9$. This result could be due to the action of natural selection on *Est-3*. It is possible that this locus was affected by natural selection. Individuals homozygous for the allele *Est-3⁹⁰* have never been found in natural populations, suggesting important differences in fitness between genotypes (Wilkins and Mathers 1973, Saavedra et al. 1993, Saavedra et al. 1995). Moreover, viability differences among genotypes of this locus were observed in the hatchery populations of Ortigueira and Ribadeo when samples of different ages were compared (Alvarez et al. 1989, Saavedra and Guerra 1996). When *Est-3* was excluded from the computation of N_e^* , a value of infinity was obtained, with a lower limit of the 95% CI of 123. It will become apparent that this result further increases the

magnitude of the differences in N_e between wild and hatchery populations.

Effective Size of Hatchery Populations

For the hatchery population of Ortigueira, the estimate of N_e obtained by the temporal method was infinity (Table 2). Because the number of oysters set to obtain this population was 60, this result has to be considered an artifact and is probably the result of the small number of loci analyzed ($n = 5$). It simply indicates that the change in allozyme frequencies observed between the two consecutive generations sampled was not large enough to be distinguished from sampling error. The other two hatchery populations gave finite estimates of N_e with the temporal method. In the case of Ribadeo, the estimate was 18.2, and the 95% CI was between 6.3 and 46.7. From the number of larvae emissions recorded, Saavedra and Guerra (1996) estimated that only 22 of the total number of 120 individuals set to spawn contributed to the offspring. There is a good agreement between this number and the estimate of N_e obtained by the temporal method. In the population of St. Vaas-le-Hougue, the estimated N_e was very low (2.4), and the 95% CI limits were 0.3 and 8.8.

The three hatchery populations suffered a loss of heterozygosity compared with the wild progenitor populations, which

TABLE 3.

Estimates of the effective population size in wild and hatchery populations of *O. edulis*.

Population	N_b	No. of Loci Scored	Heterozygosity Method				Temporal Method		
			H_o	H_t	N_e^*	95% CI	F^*	N_e^*	95% CI
Wild									
Ortigueira	~10,000	5	—	—	—	—	0.0110	248	30–∞
Hatchery									
Ortigueira	60	5	0.229 ± 0.087	0.219 ± 0.081	10.9	6.7–16.3	0.0059	∞	33–∞
Ribadeo	120	9	0.266 ± 0.054	0.233 ± 0.053	4.0	1.8–5.8	0.0370	18.2	6.3–46.7
St. Vaas-la-Hougue	Unknown	4	0.272 ± 0.096	0.170 ± 0.103	1.3	0.9–2.3	0.2131	2.4	0.3–8.8

N_b is the number of broodstock individuals used to obtain the hatchery population and the estimated census size of the population in the case of the Ortigueira wild population. H_o and H_t are average heterozygosities in wild and hatchery populations, respectively. F^* is the average across loci of the temporal variance of gene frequencies.

amounted to 4.5, 10, and 37% for Ortigueira, Ribadeo, and St. Vaas-le-Hougue, respectively. The estimates of N_e obtained from the reduction of heterozygosity were always lower than those obtained by the temporal method. The rank of the populations according to these estimates (Table 2) is the same as would be obtained from the temporal method. Ortigueira showed the highest N_e^* (10.9), followed by Ribadeo (4.0) and St. Vaas-le-Hougue (1.3) (Table 2).

In all cases, the tests for neutrality gave nonsignificant results. The number of actual remaining alleles in the populations of Ortigueira (hatchery), Ribadeo, and St. Vaas-le-Hougue were 12, 20, and 7, and the expected numbers were 12.6, 21.3, and 7.7. The differences were very small, and the highest χ^2 value obtained was 1.18 ($p = 0.281$) in St. Vaas-le-Hougue. Observed values of $nF/E(F)$ were plotted against their expectation according to the χ^2 distribution for n degrees of freedom, and no significant departures from expected distribution were found in any case.

The numbers of oysters set to spawn to obtain the hatchery populations of Ortigueira and Ribadeo were 60 and 120 (Alvarez et al. 1989, Saavedra and Guerra 1996). The comparison of these numbers with the estimated N_e pointed to important differences between the number of broodstock animals and the effective population size. The difference was of one or two orders of magnitude for the population of Ribadeo, depending on the method of estimation (temporal vs. heterozygosity). In the population of Ortigueira, although the estimate of N_e by the temporal method was infinity, the heterozygosity method gives a value one order of magnitude lower than the census size of the broodstock. No data for the size of the broodstock in the population of St. Vaas-le-Hougue are available.

DISCUSSION

Effective Size of Oyster Populations

Natural populations of bivalves normally contain a high number of individuals. However, a large disparity has been observed between the real census sizes (N) and the effective population sizes estimated from allozyme frequencies (Hedgecock et al. 1992, Hedgecock 1994). These results have placed marine bivalves among the animals with the lowest N_e/N ratio (Frankham 1995). The estimate of N_e obtained in this study for a wild population of *O. edulis* ($N_e = 248$) was also much lower than the estimated number of adult individuals in the population (~10,000). Hedgecock (1994) suggested that low N_e/N ratios could be the consequence of large variances in fertility among individuals and isolation of populations coupled with estuary retention phenomena (Hedgecock 1994). However, the estimate of N_e for the Ortigueira population is bound by a very wide confidence interval, which includes infinity as an upper limit and makes it impossible to falsify the hypothesis of larger effective sizes. Similar results have been obtained sometimes for wild populations of other oyster species (see Hedgecock et al. 1992, Table 5). Intrinsic properties of the estimation method could account for these results. Computer simulations indicate that when actual N_e is large (i.e., 25,000–75,000), an estimate using the temporal method based on 100 loci often will be finite and less than N_e with an infinite upper bound (Hedgecock and Pudovkin, Bodega Marine Laboratory, pers. comm.). The result obtained here for the wild *O. edulis* population fits that observation very well.

Other methodological problems could be influencing estimates of N_e in the wild. The temporal method of estimation used in this study was developed for a case of discrete generations (Nei and

Tajima 1981, Pollak 1983, Waples 1989). Estimates obtained from populations with overlapping generations, such as wild oyster populations, would approximate the real N_e to the extent that the samples under study constitute a random sample of all age groups in the population, provided that they were taken with an interval of more than one single generation. Otherwise, the estimate of N_e from F^* should be corrected by a factor C that depends on age-specific survival and birth rates (Jorde and Ryman 1995). Finally, it has to be noted that oyster populations are not completely closed populations. A fraction of the settling larvae are immigrants from other populations. If the immigrants differ in gene frequencies from the autoctonous individuals, then the result would be an increase in the temporal drift variance and a proportional decrease in the estimate of the effective size of the local population under study. After all of these considerations, it is clear that the estimate of N_e obtained here for the wild population of Ortigueira should be taken with caution and considered as a minimum estimate.

The temporal method of N_e estimation is suited to hatchery populations, because they are closed populations and fit the model of discrete generations. The estimates of N_e obtained by this method in the hatcheries were lower than in the wild population studied. The one exception (the hatchery population of Ortigueira) was probably the result of the small number of loci analyzed. Further, the three hatchery populations suffered a marked decrease in heterozygosity, and estimates of N_e based on this reduction support the view that they have lower effective sizes than the wild oyster bed. Previous estimates of N_e in oysters (Hedgecock and Sly 1990, Gaffney et al. 1992) also indicated that the number of animals effectively contributing to the progeny in hatchery populations was lower than the number of progenitors set to spawn. This is also true for the hatchery populations of *O. edulis* studied here. Of particular interest are the extremely low estimates of N_e in St. Vaas-le-Hougue (1.3 and 2.4), which suggest that this population could have originated from the larval emission of a single female. Implications of low effective sizes of hatchery populations will be discussed in the next section.

An interesting aspect of our results is that the estimates of N_e obtained by the heterozygosity method were always lower than those obtained from the temporal method. There has been no report comparing the results of the different methods of N_e estimation in the same population, so we cannot evaluate the generality of this observation. A possible explanation is that, although the temporal method corrects the estimate of the temporal variance of gene frequencies for sampling error, no such correction has been incorporated in our estimate of N_e from changes in heterozygosity.

Implications for Management

Consequences of low N_e in hatchery populations of *O. edulis* can appear in various ways. Responses to artificial selection indicate that *O. edulis* possesses much genetic variability for traits of economic interest, such as growth rate and resistance to parasites like *Bonamia* (Newkirk and Haley 1983, Naciri 1994). Genetic variability for the genes involved in these traits could be notably reduced in the hatchery. In addition, low N_e could give rise to inbreeding depression. Newkirk and Haley (1983) suggested that lack of response to selection for growth rate in the second generation of a hatchery line of *O. edulis* could be explained by inbreeding.

Oyster seed produced in the hatchery is usually transported to the sea for on-growing. On other occasions, it is used to replenish oyster beds that have been exhausted because of overexploitation

or diseases. In these cases, the individuals from the hatchery may contribute disproportionately to the wild population, and assuming that the wild population has a larger effective size than the hatchery populations, the consequence could be a reduction of N_e in the wild population. The release of animals obtained in captivity to replenish wild populations has been termed "supportive breeding" by Ryman and Laikre (1991), and their effects on N_e have been studied theoretically (Ryman and Laikre 1991, Ryman 1991, Ryman 1994, Ryman et al. 1995). Because the census size of the population will increase after the release of the oyster seed, one has to consider separately the effects of supporting breeding on the inbreeding generated by drift and on the drift of gene frequencies (Ryman 1994, Ryman et al. 1995). This is done by considering two kinds of effective size, namely, the inbreeding N_e (N_{ei}) and the variance N_e (N_{ev}). In most situations, N_{ei} will decrease to values below that shown by the wild population before restocking. The variance effective size could increase or decrease, depending on the values of the parameters involved (wild and hatchery populations sizes, before and after restocking), but in scenarios compatible with restocking of bivalves, it will decrease markedly. As an example, we can consider the effect of supporting breeding on the population of Ortigueira examined in this study. This population is of interest because it is representative of natural oyster beds in northwest Spain that suffered large reductions in size as the result of overfishing, diseases, and environmental degradation and that are being taken into consideration for recovery plans (Saavedra et al. 1987, Consellería de Pesca 1993). The census size of the wild Ortigueira population was approximately $n = 10,000$ (although our estimate of the effective size is 248), and a sample of 60 oysters was taken to use as broodstock in the hatchery. Suppose that, in the context of a recovery plan, we want to double the size of the population. If we assume that the wild population remains constant in size and that all of the oyster seed will survive when transferred to the wild, then 10,060 oyster seed from the hatchery (with an effective size of 10.9) have to be released to get a final population size of 20,000. By using equation (1) in Ryman et al.

(1995), we get a value $N_{ev} = 29.7$, which is almost one-tenth of the effective size of the wild population with no supporting breeding. The inbreeding N_e can be obtained from equation (1) in Ryman and Laikre (1991) and turns out to be $N_{ei} = 41.8$. This means that, in one single generation, a reduction of N_e of more than 80% could result, and the inbreeding rate could increase more than fivefold.

As stated by Ryman (1991), supportive breeding results in a trade-off, in which the economical gain is associated with a loss of genetic variability. The increase in production usually relegates concerns about conservation of genetic variability to a secondary place. In fact, a large reduction of N_e may be not important for a population, especially when N_e and reproductive potential are large. However, the effective population sizes of bivalve species may be not as large as one could suppose from the real census sizes (Hedgecock et al. 1992, Hedgecock 1994, David and Jarne 1997, this study). Therefore, the continued introduction of hatchery oyster seed into wild populations during a period of several years could be risky, especially if the progenitors are always taken from the same population. In this case, the increase of average inbreeding could be an issue of concern. Management policies that favor supportive breeding for replenishment of exhausted oyster beds, or large-scale models of exploitation based in small hatcheries for local production of seed and growout, (Consellería de Pesca 1993) should be planned carefully and must include monitoring of the wild and hatchery populations to avoid damage to genetic resources.

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GROWTH AND FATTY ACID COMPOSITION OF PACIFIC OYSTER (*CRASSOSTREA GIGAS*) SPAT FED A MICROALGA AND MICROCAPSULES CONTAINING VARYING AMOUNTS OF EICOSAPENTAENOIC AND DOCOSAHEXAENOIC ACID

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ABSTRACT Pacific oyster (*Crassostrea gigas*) spat were fed for 28 days on either a 100% ration of the microalga *Dunaliella tertiolecta*, which lacks fatty acids greater than C₁₈, or an 80% ration of *D. tertiolecta* and 20% gelatin-acacia microcapsules (GAM). GAM contained corn oil alone or corn oil supplemented with varying amounts of either eicosapentaenoic acid (20:5n-3, EPA), docosahexaenoic acid (22:6n-3, DHA), or combinations of the two. GAM containing either corn oil, corn oil containing up to 0.16% EPA (dry weight of GAM), 0.63% DHA, or 0.32% of an EPA/DHA mixture did not improve shell length, dry weight, or ash-free dry weight (AFDW) of spat compared with spat fed *D. tertiolecta* alone. However, GAM containing 0.30 and 0.50% EPA resulted in spat with significantly higher AFDW than spat fed either *D. tertiolecta* alone or *D. tertiolecta* plus GAM containing corn oil. There was a significant positive correlation between the level of EPA present in GAM and AFDW of spat. The results suggested that spat growth may improve further at levels of dietary EPA higher than those used in this study. The fatty acid profile of spat generally reflected that of the diet after 28 days. However, the increase in dietary levels of both EPA and DHA were not reflected and unfed spat selectively retained EPA and DHA.

KEY WORDS: *Crassostrea gigas*, DHA, EPA, microcapsules, nutrition, oysters

INTRODUCTION

It is now well-established that *n*-3 highly unsaturated fatty acids (HUFAs) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are essential dietary components for marine fish (Bell et al. 1986, Sargent et al. 1989, Ibeas et al. 1994), crustaceans (Kanazawa et al. 1979a, Kanazawa et al. 1979b, Castell 1983), and molluscs (Langdon and Waldock 1981, Uki et al. 1986, Chu and Greaves 1991).

Although some bivalve molluscs have a very low capacity to elongate and desaturate linolenic acid (18:3n-3) to C₂₀ and C₂₂ HUFAs (De Moreno et al. 1976, Waldock and Holland 1984, Chu and Greaves 1991), it is not sufficient to support optimal growth. A number of studies have been undertaken to define the HUFA requirements of bivalves. In some of these studies, bivalves have been fed various species or strains of microalgae with different fatty acid (FA) contents (Enright et al. 1986a, Helm and Laing 1987, Delaunay et al. 1993, Thompson et al. 1993, Albentosa et al. 1994, Albentosa et al. 1996, Vanderploeg et al. 1996, Wikfors et al. 1996). Other studies have used single species of microalgae, grown under different conditions, to produce diets varying in their FA composition (Enright et al. 1986b, Thompson and Harrison 1992, Thompson et al. 1996). Limitations to this type of experiment include factors such as interspecific differences in digestibility and differences in nutritional components other than FAs, which may influence the results obtained (Webb and Chu 1983).

Ideally, nutritional requirements of bivalves should be determined using complete artificial diets, the composition of which can be precisely controlled. In the absence of such diets, FA requirements have also been investigated with microalgae lacking FAs greater than C₁₈ supplemented with gelatin-acacia microcapsules (GAM) containing lipids of different FA compositions (Langdon and Waldock 1981, Knauer and Southgate 1997a). Using this technique, the essentiality of *n*-3 HUFAs for Pacific oyster (*Crassostrea gigas*) spat has been demonstrated (Langdon and

Waldock 1981, Knauer and Southgate 1997a); this technique would seem to be a promising approach to further studies on the lipid requirements of bivalves. GAM are readily digested by bivalves (Chu et al. 1982, Southgate 1988), and lipid supplied in GAM is assimilated with high efficiency (Knauer and Southgate 1997b). GAM have been used successfully to present dietary lipids in a number of studies with bivalves (Langdon and Waldock 1981, Chu et al. 1982, Chu et al. 1987, Southgate 1988, Numaguchi and Nell 1991, Knauer and Southgate 1997a).

It has been suggested that either EPA or DHA alone can satisfy the *n*-3 HUFA requirement of bivalves (Langdon and Waldock 1981). Therefore, this study investigated the relative importance of EPA and DHA for *C. gigas* spat, using a microalga lacking FAs greater than C₁₈ supplemented with GAM containing different amounts of EPA and/or DHA.

MATERIALS AND METHODS

Diets

The marine flagellate *Dunaliella tertiolecta* (code CS 175) was obtained from CSIRO Marine Laboratories, Hobart, Australia. Cultures were grown in 10-L carboys with f/2 medium without silicate (Guillard 1975) and maintained at 25.2 ± 1.3°C under a 12-h light:12-h dark photoperiod. Cultures in the exponential growth phase were used to feed spat, and each day, all spat were fed with *D. tertiolecta* from the same hatch culture. The dry weight (DW) of *D. tertiolecta* was determined as 115.62 ± 3.87 pg cell⁻¹ by the method of Utting (1985).

GAM were prepared according to the method of Southgate and Lou (1995) with 2.5 mL of corn oil and 2.5 mL of corn oil supplemented with either 5, 25, 50, or 75 mg of EPA (E7006, Sigma) or DHA (D2534, Sigma) or mixtures of both FAs (EPA/DHA: 12.5/

12.5, 20/5, 5/20 mg). The diameter of GAM containing corn oil was $4.6 \pm 1.2 \mu\text{m}$ ($n = 100$). Stock suspensions of GAM were kept at 4°C and were shaken daily. The DW of GAM per unit stock suspension was determined by oven-drying triplicate 1-mL volumes of stock suspension.

The caloric content of each type of GAM was determined in triplicate following the method of Knauer and Southgate (1997a). Oven-dried (60°C) samples of GAM (86.1–214.3 mg) were used, and a standard curve was generated using benzoic acid (48.7–539.1 mg).

Feeding Experiment

Hatchery-reared *C. gigas* spat were obtained from Shellfish Culture P/L, Tasmania, Australia. They were unfed for 1 day, after which 50 randomly selected spat were used to determine the initial shell length (SL), DW, and ash-free dry weight (AFDW). Another 50 spat were washed with distilled water and a 2:1 (v/v) chloroform/methanol mixture (Langdon and Waddock 1981) and then frozen at -80°C for analysis of their initial FA composition.

Each aquarium was stocked with 50 spat held in plastic mesh (pore size, 1-mm diameter) baskets. Group wet weights of spat ranged from 1.31 to 1.40 g, with the variation from the mean wet weight of all groups ($1.37 \pm 0.03 \text{ g}$) not exceeding $\pm 5\%$. Spat were kept under a 12-h light:12-h dark photoperiod, and seawater temperature was maintained at $24.9 \pm 0.7^\circ\text{C}$. Seawater was filtered through cartridge filters with pore sizes of 5, 1 and $0.45 \mu\text{m}$, and an activated carbon cartridge filter and then ultraviolet sterilized before use. Each aquarium was filled with 4 L of filtered seawater (FSW) with a salinity of 30‰ and was gently aerated to reduce food sedimentation. FSW in all aquaria was changed every 24 h using a flow-through system, and each aquarium was sterilized with chlorine solution and washed with freshwater every 5 days. At the same time, baskets and spat were cleaned by spraying with FSW.

The experiment was randomized with three replicates per treatment. Each aquarium received the same DW ration once daily, which was calculated using the formula of Epifanio (1979):

$$Q_R = 0.01 \times W^{-0.33}$$

where Q_R = the DW of ration per g wet weight of oysters, and W = g initial wet weight of oysters. Spat were fed either 100% *D. tertiolecta* or an 80% ration of *D. tertiolecta* supplemented with 20% GAM. Spat in a further three aquaria remained unfed throughout the experiment. After 28 days, all animals were unfed for 1 day to clear their gut contents. After that, 20 animals from each aquarium were sampled for measurement of SL, DW, and AFDW, and the remaining spat from two of the three treatment replicates were pooled and processed for determination of FA profiles as above.

FA Analyses and Growth Measurements

A 1-L sample from each of two batches of *D. tertiolecta* was centrifuged at $3,000 \text{ g}$ for 10 min, washed with 100 mL of 0.5 M ammonium formate, and recentrifuged (Brown and Jeffrey 1992). The resulting pastes were stored under nitrogen at -80°C . A sample of each type of GAM, taken halfway through the growth trial (after 14 days), was stored in the same way before FA analysis.

The lipid fractions of spat and the *D. tertiolecta* pastes were

extracted for FA analysis following the method of Dunstan et al. (1993), whereas GAM were directly processed. FA methyl esters (FAMES) were prepared according to the method of Dunstan et al. (1993), dried under nitrogen, and stored at -80°C before analysis. Controls containing known amounts of DHA were run simultaneously to determine the efficiency of the derivatization process. FAMES were analyzed with a gas chromatograph and a mass selective detection analyzer following the method of Knauer and Southgate (1997a). Nonadecanoic acid (19:0) was used as an internal standard for quantitative determinations. The SL, DW, and AFDW of spat were determined as described previously (Knauer and Southgate 1996).

Statistical Analyses

The homogeneity of the variances of means was analyzed using Cochran's test. Logarithmic transformations of data were performed if the assumptions of analysis of variance were not fulfilled. The results of the caloric contents of GAM and the growth data were analyzed using a one-way analysis of variance. Multiple comparisons were made using Tukey's multiple range test. Results were considered to be significantly different at $p \leq 0.05$.

RESULTS

The concentrations of EPA and DHA in GAM are shown in Table 1. The addition of various amounts of EPA resulted in GAM with an EPA content ranging from 0.04 to 0.50% of the DW of GAM. The total amount of EPA fed daily per replicate varied from 1.34 to 19.70 μg . Similarly, adding various amounts of DHA produced GAM containing 0.04–0.63% DHA. However, the addition of 50 mg of DHA resulted in a higher content (0.63%) than did the addition of 75 mg (0.57%). The total amount of DHA fed daily per replicate varied from 1.40 to 25.29 μg . The addition of a 1:1 mixture of both FAs resulted in an EPA content of 0.14% and a DHA content of 0.18% DW. When added in a 4:1 or 1:4 ratio, GAM contained 0.19% EPA and 0.06% DHA or 0.05% EPA and

TABLE 1.
Total amount of EPA and/or DHA added to 2.5 mL of corn oil before microencapsulation, their content in GAM, and the amount fed daily to *C. gigas* spat.

Total Amount Added (mg)	% DW of GAM	Amount Fed Daily (μg) per Replicate*
EPA		
5.0	0.04	1.34
25.0	0.16	6.38
50.0	0.30	12.00
75.0	0.50	19.70
DHA		
5.0	0.04	1.40
25.0	0.19	7.56
50.0	0.63	25.29
75.0	0.57	22.71
EPA/DHA		
12.5/12.5	0.14/0.18	5.68/7.03
20.0/5.0	0.19/0.06	7.49/2.57
5.0/20.0	0.05/0.16	1.71/6.21

* GAM accounted for 20% of the DW ration.

0.16% DHA, respectively. The amount of the FA mixtures fed daily per replicate ranged from a total of 7.92 to 12.71 μg .

The energy content of the 12 types of GAM ranged from $36.91 \pm 0.63 \text{ J mg}^{-1}$ in GAM containing 0.30% EPA to $37.80 \pm 0.16 \text{ J mg}^{-1}$ in GAM containing 0.16% EPA and $37.80 \pm 0.19 \text{ J mg}^{-1}$ in GAM containing 0.57% DHA (Table 2). However, these differences were not significant.

The major FA compositions of *D. tertiolecta* and GAM containing various amounts of EPA and DHA are shown in Table 3. The FA profile of *D. tertiolecta* was dominated by 18:3n-3 (49.1 \pm 2.8%) and 16:0 (31.2 \pm 1.8%), and no FA larger than C_{18} was detected. Microencapsulated corn oil contained high levels of 18:2n-6 (49.6%) and 18:1n-9 (21.9%) and again did not contain any FA larger than C_{18} . In GAM supplemented with EPA, the level of EPA increased from 13.1 to 73.1%. The level of DHA in GAM ranged from 13.3 to 78.5%, however, the relative level was highest at a DHA content of 0.57% (DW of GAM) rather than at 0.63%. Similarly, when both EPA and DHA were added, the % level of DHA in GAM was higher at the 0.16% inclusion level than at the 0.18% level.

Mortalities varied from 0 to 4% per replicate with no significant difference between treatments. The SL, DW, and AFDW of *C. gigas* spat fed *D. tertiolecta* plus GAM containing various amounts of EPA and DHA are presented in Table 4. All fed spat had a significantly greater SL, DW, and AFDW than did unfed spat, but there were no significant differences in SL and DW between spat in any fed treatment. However, the AFDW of spat fed *D. tertiolecta* plus GAM containing 0.50% EPA ($1.77 \pm 0.04 \text{ mg}$) was significantly greater than that of spat fed the other GAM-substituted diets, with the exception of spat fed *D. tertiolecta* plus GAM containing 0.30% EPA ($1.51 \pm 0.03 \text{ mg}$). Moreover, spat fed *D. tertiolecta* and either GAM containing up to 0.16% EPA, 0.63% DHA, or 0.32% EPA/DHA mixtures were not significantly different from spat fed either *D. tertiolecta* alone ($1.20 \pm 0.10 \text{ mg}$) or *D. tertiolecta* plus GAM containing corn oil alone ($1.16 \pm 0.04 \text{ mg}$). There was no relationship between the energy contents of the experimental GAM and the resulting AFDW of spat (EPA: $r^2 = 0.0120$, $p \geq 0.05$; DHA: $r^2 = 0.0026$, $p \geq 0.05$).

The predominant FAs of the initial sample of *C. gigas* spat

were EPA (21.5 \pm 1.8%), DHA (23.3 \pm 3.0%), and 16:0 (20.1 \pm 4.2%) (Table 5). All fed spat showed an increase in the relative content of 14:0, 18:1n-9, 18:2n-6, and 18:3n-3 at the end of the 28-day growth trial. In contrast, all fed spat contained reduced levels of EPA (2.5–6.1%) and DHA (3.8–9.6%) compared with the initial FA profile. At the end of the growth trial, unfed spat contained relatively high EPA (8.0 \pm 3.5%) and DHA (14.7 \pm 2.7%) levels compared with fed spat and showed increases in the contents of 12:0, 14:0, 18:1n-7, 20:1n-7, and arachidonic acid (20:4n-6).

There was a significant positive correlation between the dietary EPA content and AFDW of spat fed GAM containing EPA ($r^2 = 0.8977$, $p \leq 0.02$) (Fig. 1). No such relationship was found for dietary DHA ($r^2 = 0.4623$, $p \geq 0.05$).

DISCUSSION

Microcapsules have been shown to be a promising research tool to determine the nutritional requirements of bivalves. For example, cross-linked protein-walled microcapsules have been used to investigate the protein requirements of bivalves (Kreeger and Langdon 1993). This study demonstrated the suitability of GAM as a means to quantitate the HUFA requirements of bivalves. The FA content of GAM can be easily manipulated, and in contrast to microalgae, there are no other dietary variables that could influence growth rates during nutritional experiments. A potential problem in nutritional studies using microcapsules is the possibility of bacteria, present in seawater or associated with the microcapsules themselves, influencing bivalve growth (Langdon and Bolton 1984, Langdon and DeBevoise 1990). However, because most marine bacteria do not contain HUFAs (Kaneda 1967, Hayashi and Takagi 1977, Brown et al. 1996), they are unlikely to pose a problem for studies using GAM to quantitate HUFA requirements.

Langdon and Waldo (1981) were the first to establish the essentiality of n-3 HUFAs for bivalves. More recently, the AFDW of *C. gigas* spat was shown to be positively correlated with the levels of EPA and DHA present in GAM (Knauer and Southgate 1997a). Because both FAs were offered simultaneously, however, the relative importance of EPA and DHA could not be determined (Knauer and Southgate 1997a). In this study, only spat fed GAM containing either 0.30 or 0.50% EPA had a significantly greater AFDW than did spat fed *D. tertiolecta* alone or *D. tertiolecta* plus GAM containing corn oil. GAM accounted for 20% of the diet, and it has previously been shown that, under identical experimental conditions, 26% of dietary GAM presented to *C. gigas* spat are ingested (Knauer and Southgate 1997b). Assuming a similar level of ingestion in this study, it is likely that the content of EPA resulting in improved spat growth was in the range of 0.02–0.03% (DW of diet).

It has been suggested that in oysters, there is a threshold level for dietary essential FAs beyond which further increases do not improve growth rate (Thompson and Harrison 1992). It is likely that the highest level of dietary EPA used in this study fell below any such threshold, because the correlation between % EPA in GAM and spat AFDW suggests that higher dietary EPA contents may further increase spat growth.

In contrast to EPA, there was no correlation between the content of DHA in GAM, which ranged from 0.04 to 0.63%, and AFDW of spat. Similarly, a wide range of DHA levels (1.9–12% of total FAs) in microalgae has also been found to have no apparent influence on the growth rate of *C. gigas* larvae (Thompson et al. 1993). On the other hand, increases in the DHA content of

TABLE 2.

Energy contents (J mg^{-1}) of GAM containing corn oil plus various amounts of EPA and/or DHA.

% DW of GAM	J mg^{-1}
Corn oil	37.41 ± 0.08
Corn oil + EPA	
0.04	37.27 ± 0.21
0.16	37.80 ± 0.16
0.30	36.91 ± 0.63
0.50	37.63 ± 0.04
Corn oil + DHA	
0.04	37.52 ± 0.04
0.19	36.97 ± 0.64
0.57	37.80 ± 0.19
0.63	37.47 ± 0.00
Corn oil + EPA/DHA	
0.14/0.18	37.47 ± 0.01
0.19/0.06	37.59 ± 0.66
0.05/0.16	37.38 ± 0.31

Values are the mean \pm SD ($n = 3$).

TABLE 3.

Major FA composition (>1% of total FA) of *D. tertiolecta* and GAM containing corn oil plus various amounts of EPA and/or DHA.

FA	<i>D. tertiolecta</i> *	Corn Oil	% DW GAM										
			EPA				DHA				EPA/DHA		
			0.04	0.16	0.30	0.50	0.04	0.19	0.57	0.63	0.14/0.18	0.19/0.06	0.05/0.16
14:0	—	1.0	—	—	—	—	—	—	—	—	—	—	—
16:0	31.2 ± 1.8	12.3	16.1	11.2	6.5	4.1	15.6	7.5	3.8	5.4	9.0	10.1	11.9
18:0	—	1.7	2.9	1.7	—	—	—	—	—	—	—	—	—
18:1 <i>n</i> - 7	1.6 ± 0.1	—	—	—	—	—	—	—	—	—	—	—	—
18:1 <i>n</i> - 9	3.2 ± 0.2	21.9	21.8	14.0	9.0	5.9	22.4	12.0	5.5	7.2	11.1	13.3	14.3
18:2 <i>n</i> - 6	5.1 ± 0.3	49.6	46.1	31.2	18.6	12.0	48.7	27.4	12.3	15.7	25.0	29.4	33.8
18:3 <i>n</i> - 3	49.1 ± 2.8	2.7	—	2.0	3.8	5.0	—	1.0	—	—	1.0	1.4	—
20:5 <i>n</i> - 3	—	—	13.1	37.2	62.2	73.1	—	—	—	—	24.1	34.0	8.8
22:6 <i>n</i> - 3	—	—	—	—	—	—	13.3	51.1	78.5	71.7	29.9	11.8	31.2

* Values are the mean ± range (n = 2).

microalgae have been shown to enhance growth rates of bivalves (Enright et al. 1986b, Thompson and Harrison 1992). Enright et al. (1986b) fed *Chaetoceros muelleri* containing 0.08 to 0.23% DHA (dry weight of diet) to juvenile *Ostrea edulis*, and at a level of 0.12%, DHA was suggested to be a growth-limiting component (Enright et al. 1986b). In this study, assuming that 26% of dietary GAM were ingested, the highest content of DHA fed was 0.03% (DW of diet), which is considerably lower than the level of DHA proposed to be growth-limiting in *O. edulis* (Enright et al. 1986b).

GAM containing mixtures of EPA/DHA (up to a total of 0.32% DW of diet) did not improve the AFDW of spat compared with AFDW of those fed either *D. tertiolecta* alone or *D. tertiolecta* plus GAM containing corn oil. A level of at least 0.30% EPA in

GAM was required to improve spat growth in this study. Therefore, it is not surprising that GAM containing EPA/DHA mixtures, where EPA levels did not exceed 0.19%, did not improve growth. However, dietary EPA/DHA ratios may be important for bivalves at higher levels of supplementation. Dietary EPA/DHA ratio has been shown to influence growth rates of crustaceans (Kontara et al. 1995, Naessens et al. 1995) and fish (Kalogeropoulos et al. 1992, Ibeas et al. 1994).

Currently, data regarding the relative importance of EPA and DHA for bivalves are contradictory. A specific requirement for EPA by some oyster larvae and spat has been demonstrated (Helm and Laing 1987, Wikfors et al. 1996) that supports the results of this study. In contrast, the importance of DHA has been emphasized for oyster juveniles (Enright et al. 1986b), clam larvae and spat (Helm and Laing 1987, Albentosa et al. 1994), and scallop larvae and juveniles (Delaunay et al. 1993, Coutteau et al. 1996). It has also been reported that growth of clam spat was not limited by the absence of either EPA or DHA (Albentosa et al. 1996), and some clam larvae do not appear to have a requirement for *n*-3 HUFAs at all (Laing et al. 1990). Although current data indicate that HUFA requirements differ between bivalve species, it is possible that differences in experimental parameters, such as temperature, influenced the results of these studies. For example, growth rates of greenlip abalone (*Haliotis laevis*) juveniles fed diets containing elevated levels of EPA were higher than those of juveniles fed diets containing less EPA at water temperatures of 10.1–13.1°C, but not at water temperatures of 10.1–17.6°C (Dunstan et al. 1996).

The major FA compositions previously reported for corn oil (Knauer and Southgate 1997a) and *D. tertiolecta* (Langdon and Waldock 1981, Delaunay et al. 1993) are similar to those reported in this study. Interestingly, EPA and DHA accounted for only up to 0.63% of the dry weight of GAM, but made up a very high proportion of the total FAs of the GAM which contained mainly corn oil. The reason for this deviation in the expected FA profile of the GAM is unclear; however, a similar deviation has previously been reported during microencapsulation of a 1:1 mixture of corn oil and squid oil (Knauer and Southgate 1997a). The FA profiles of all fed spat at the end of the growth trial reflected the high 18:3n-3 content of *D. tertiolecta* and the high 18:2n-6 content of corn oil. This confirms previous findings that the FA composition of *C. gigas* tissues is influenced by that of the diet (Waldock and

TABLE 4.

SL, DW, and AFDW of *C. gigas* spat fed *D. tertiolecta* (DT) and GAM containing corn oil (COR) supplemented with different amounts of EPA and/or DHA for 28 days.

Diet	SL (mm)	DW (mg)	AFDW (mg)
Unfed	5.21 ± 0.11 ^b	14.17 ± 0.23 ^b	0.66 ± 0.04 ^d
DT	7.16 ± 0.37 ^a	19.05 ± 1.36 ^a	1.20 ± 0.10 ^c
DT + (COR)	7.36 ± 0.33 ^a	19.14 ± 1.68 ^a	1.16 ± 0.04 ^c
DT + (COR + % EPA)			
0.04	7.33 ± 0.50 ^a	19.81 ± 1.45 ^a	1.39 ± 0.08 ^{b,c}
0.16	7.53 ± 0.27 ^a	20.67 ± 1.55 ^a	1.41 ± 0.18 ^{b,c}
0.30	7.49 ± 0.22 ^a	20.53 ± 1.90 ^a	1.51 ± 0.03 ^{a,b}
0.50	7.33 ± 0.34 ^a	20.66 ± 0.96 ^a	1.77 ± 0.04 ^a
DT + (COR + % DHA)			
0.04	7.29 ± 0.37 ^a	19.41 ± 1.55 ^a	1.36 ± 0.06 ^{b,c}
0.19	7.30 ± 0.12 ^a	19.12 ± 0.88 ^a	1.37 ± 0.06 ^{b,c}
0.57	7.29 ± 0.15 ^a	19.41 ± 0.96 ^a	1.37 ± 0.07 ^{b,c}
0.63	7.40 ± 0.19 ^a	19.74 ± 1.04 ^a	1.43 ± 0.19 ^{b,c}
DT + (COR + % EPA/DHA)			
0.14/0.18	7.45 ± 0.14 ^a	19.94 ± 0.27 ^a	1.32 ± 0.05 ^{b,c}
0.19/0.06	7.30 ± 0.17 ^a	19.14 ± 0.84 ^a	1.34 ± 0.07 ^{b,c}
0.05/0.16	7.36 ± 0.21 ^a	19.38 ± 1.05 ^a	1.39 ± 0.06 ^{b,c}

Initial values (n = 50): SL 5.11 ± 0.52 mm; DW, 14.00 ± 3.78 mg; AFDW, 0.79 ± 0.25 mg. Values are the mean ± SD (n = 3). Means in each column with different superscripts are significantly different (p ≤ 0.05).

TABLE 5.

Major FA composition (>1% of total FA) of *C. gigas* spat fed *D. tertiolecta* (DT) and GAM containing corn oil (COR) plus different amounts of EPA and/or DHA for 28 days.

FA	Initial	Unfed	DT	DT+ (COR)	DT + (COR + % DW GAM)										
					EPA				DHA				EPA/DHA		
					0.04	0.16	0.30	0.50	0.04	0.19	0.57	0.63	0.14/0.18	0.19/0.06	0.05/0.16
12:0	—	8.0 ±0.1	—	8.7 ±2.1	4.6 ±0.9	2.4 ±0.2	2.6 ±1.1	2.2 ±1.0	2.3 ±0.0	5.0 ±1.0	3.1 ±0.9	5.4 ±2.7	1.0 ±0.4	—	5.8 ±1.1
14:0	—	10.8 ±1.7	4.9 ±1.3	13.7 ±0.3	3.3 ±1.0	1.2 ±0.0	3.1 ±1.9	5.3 ±1.6	4.8 ±2.1	1.2 ±0.3	7.4 ±1.8	5.1 ±1.5	3.4 ±0.1	2.9 ±1.4	4.2 ±1.3
16:0	20.1 ±4.2	18.6 ±0.7	27.5 ±4.1	18.2 ±2.7	22.0 ±0.1	21.1 ±3.1	20.8 ±0.9	17.5 ±3.0	24.9 ±1.6	28.2 ±3.0	15.6 ±3.1	19.3 ±2.3	23.6 ±4.2	28.7 ±3.3	23.7 ±3.2
16:4n-1	—	—	1.1 ±0.6	1.1 ±0.4	—	—	1.6 ±0.8	—	—	1.1 ±2.1	4.7 ±2.6	3.3 ±1.6	—	2.0 ±1.0	—
18:0	8.2 ±2.4	8.7 ±1.9	7.9 ±1.4	7.4 ±1.6	3.0 ±0.3	9.8 ±2.1	7.3 ±0.2	3.9 ±2.3	9.4 ±0.8	6.4 ±0.6	4.8 ±2.6	9.6 ±2.9	5.2 ±1.9	10.5 ±2.6	7.6 ±1.8
18:1n-7	2.2 ±1.4	7.5 ±1.2	1.0 ±1.0	—	3.1 ±0.6	1.0 ±0.2	3.9 ±0.2	2.3 ±0.4	—	—	4.3 ±1.4	3.3 ±0.9	—	2.1 ±2.4	—
18:1n-9	4.6 ±1.2	2.0 ±0.4	10.1 ±2.7	9.2 ±2.6	9.1 ±3.1	11.3 ±0.5	13.4 ±3.2	13.0 ±4.1	7.1 ±0.2	5.5 ±0.9	11.8 ±3.8	10.4 ±2.4	15.2 ±2.4	9.2 ±1.7	14.2 ±2.7
18:2n-6	4.5 ±0.9	4.3 ±2.0	7.3 ±1.3	16.8 ±2.7	22.5 ±3.0	16.1 ±2.1	19.9 ±4.5	24.8 ±2.8	18.1 ±2.5	15.9 ±2.1	14.3 ±2.4	14.7 ±4.2	19.2 ±2.0	16.8 ±3.8	19.9 ±3.8
18:3n-3	4.2 ±0.7	2.3 ±1.4	16.2 ±1.0	14.6 ±2.0	12.3 ±1.2	16.2 ±2.6	12.7 ±1.2	11.7 ±2.1	6.9 ±0.3	11.9 ±1.0	12.2 ±0.5	6.1 ±2.7	9.0 ±3.1	8.3 ±0.5	12.4 ±0.8
20:1n-7	—	2.1 ±0.7	—	—	—	—	—	—	—	1.5 ±0.9	—	3.3 ±2.4	—	—	—
20:4n-6	2.3 ±2.1	6.1 ±1.1	2.4 ±0.4	1.2 ±0.7	1.0 ±0.5	4.4 ±1.2	2.7 ±0.2	1.4 ±0.4	4.4 ±1.4	4.2 ±1.2	5.0 ±1.9	4.3 ±1.7	3.2 ±1.2	3.3 ±0.7	1.3 ±0.1
20:5n-3	21.5 ±1.8	8.0 ±3.5	4.1 ±1.1	2.5 ±1.0	6.1 ±1.6	4.6 ±1.8	4.0 ±0.9	5.4 ±0.6	4.5 ±1.3	3.6 ±1.4	5.5 ±2.8	4.3 ±2.0	4.2 ±0.2	5.3 ±0.7	2.5 ±0.3
22:6n-3	23.3 ±3.0	14.7 ±2.7	9.1 ±3.1	3.8 ±0.0	4.8 ±1.7	8.4 ±1.2	5.3 ±1.7	5.5 ±0.4	9.6 ±0.9	5.8 ±0.8	3.9 ±1.5	5.2 ±3.0	8.2 ±2.3	4.6 ±1.3	5.2 ±0.3

Values are the mean ± range (n = 2).

Nascimento 1979, Langdon and Waldoock 1981, Knauer and Southgate 1997a). However, there was no correlation between the increases in the relative levels of EPA and DHA in GAM and the FA profiles of spat.

Unfed spat showed an increase in the % levels of 12:0, 14:0,

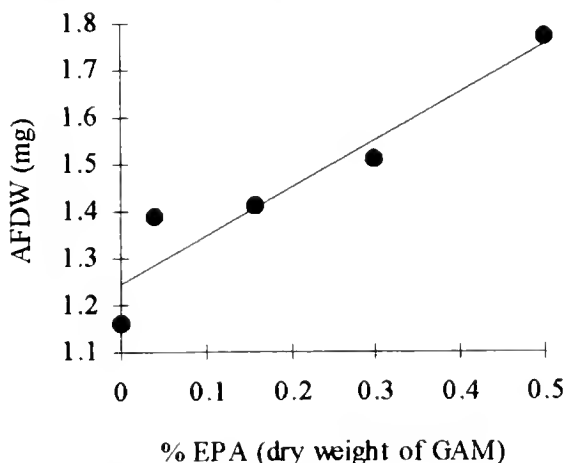


Figure 1. Positive correlation between dietary content of EPA and AFDW of *C. gigas* spat fed *D. tertiolecta* and GAM containing various amounts of EPA ($r^2 = 0.8977$, $p \leq 0.02$).

18:1n-7, 20:1n-7, and 20:4n-6 compared with initial spat. The increase in saturated FAs may have been due to uptake of bacteria or dissolved organic matter from seawater (Langdon and Waldoock 1981). An increase in tissue levels of 18:1n-7 and 20:1n-7 has also been detected in nutritional studies in fed and unfed *C. gigas* larvae (Thompson and Harrison 1992, Thompson et al. 1993) and spat (Knauer and Southgate 1997a). The exact function of these FAs is not known, but they are probably intermediates in the synthesis of C_{22} nonmethylene interrupted FAs (Whyte 1988, Thompson and Harrison 1992). The level of DHA in unfed spat was higher than the EPA level after 28 days, which supports the findings of similar studies (Coutteau et al. 1996, Knauer and Southgate 1997a). This has been interpreted as indicating a more important role for DHA than EPA in bivalve spat (Coutteau et al. 1996). However, the results of this study indicate that for *C. gigas* spat, EPA is a more important dietary component than DHA.

In conclusion, this study demonstrated a positive correlation between dietary EPA content and AFDW of *C. gigas* spat. Spat growth was improved significantly at dietary levels of 0.30 and 0.50% EPA in GAM. It is likely, however, that spat growth would further increase at higher levels of EPA supplementation. The levels of dietary DHA and EPA/DHA ratios used in this study did not have a significant effect on spat growth, and their respective roles remain unclear. Further studies are required to define optimal dietary levels of $n-3$ HUFAs for *C. gigas* and the importance of the

EPA/DHA ratio. This is the first study to attempt to quantify the *n*-3 HUFA requirements of bivalves using precisely manipulated diets. Although the results are of a preliminary nature, they provide a useful benchmark for future studies in this field.

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GROWTH AND BIOCHEMICAL COMPOSITION OF *CRASSOSTREA GIGAS* (THUNBERG) AT THREE FISHFARM EARTHEN PONDS

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ABSTRACT Growth and survival of *Crassostrea gigas* (Thunberg) juveniles, from natural spatfall, were compared at three earthen fishponds in the northwestern Portuguese coast, from June 1990 to October 1991. Station Marinha was located at Ria de Aveiro, a seawater lagoon with little incoming freshwater. Stations INIP and Gil, located at Mondego River estuary, experienced an important influence from freshwater, particularly Station Gil. Oysters were sampled at 45-day intervals for their growth, condition index, and biochemical composition. Temperature, salinity, and particulate organic and inorganic matter in the water at the test sites were monitored at the same time. Juveniles initially with 0.4 g reached the end of the experimental period with an average of 53, 50, and 38 g at Stations Marinha, INIP and Gil, respectively. In general, oysters from Station Gil showed the worst condition, especially during the second growing season. Oysters from both Mondego stations presented shells infested by *Polydora* sp. Apparently, the high mortalities observed at the same stations had nothing to do with the worm infestation. Mortality at Station Marinha was negligible. Variations observed in dry meat weight and lipid and carbohydrate levels are probably associated with gonad growth and spawning activity at Stations Marinha and INIP. Several factors that may have been responsible for the observed differences in oyster performance are discussed. In particular, the water movement is thought to have played a key role in dictating oyster performance. Fish farm earthen ponds, with controlled water circulation, seem to be suitable sites for intermediary oysters growth.

KEY WORDS: *Crassostrea gigas*, *Polydora* sp., biochemical composition, Ria de Aveiro, Mondego estuary

INTRODUCTION

The River Mondego estuary and Ria de Aveiro lagoon (Portugal) have a large area of salt marshes, some of which are still being used for salt production. In the last few years, however, because of difficulties in the salt industry and the growing interest in aquaculture, part of the producers are converting salt ponds into fish ponds. The species grown at these places are *Sparus aurata*, *Dicentrarchus labrax*, *Anguilla anguilla*, and *Mugil cephalus*.

The water circulation in the ponds depends on tide levels. In the tanks built at a higher level, a pumping system is needed to assure a convenient water supply in the lower tides, particularly during the summer.

Several years ago, various beds of the European oyster (*Ostrea edulis*) could be found at these places, as proved by the amount of shells that can now be seen in some areas. Because the oyster is a sessile and filter-feeding species that does not compete with any of the fish species produced, it seemed useful to investigate the growth and condition index of oysters reared in the inlet and outlet channels of some fish farms at these places. The great variations in temperature and salinity in these pond systems encourage consideration of the use of *Crassostrea* sp. instead of *Ostrea* sp.

Crassostrea gigas (Thunberg), the Pacific oyster, is being grown in increasing numbers throughout the world. The attraction for cultivating this species arises from its efficiency as a filter feeder, its fast growth rate, and its reported tolerance to a wide range of physical conditions such as temperature, salinity, and silt load in the water (Quayle 1969, Bardach et al. 1972, Shpigel and Blaylock 1991). It is also less susceptible to disease than *O. edulis*.

Experiments with the purpose of using *C. gigas* on polyculture systems (Hughes-Games 1977, Coeurdacier et al. 1983, Jones and Iwama 1991) or as a biological filter on integrated fish/bivalve cultures (Shpigel and Blaylock 1991; Shpigel et al. 1993), have already been described elsewhere. This study describes the grow-

ing performance of *C. gigas*, as well as condition indices and levels of storage products of these oysters, reared in three fish farms in two estuaries from the north of Portugal, between June 1990 and October 1991. Because local variations in water quality can significantly affect the productivity of the Pacific oyster in coastal areas (Héral et al. 1984, Héral et al. 1987, Brown and Hartwick 1988), water temperature, salinity, and total particulate matter were also measured.

MATERIALS AND METHODS

Study Areas

This experiment took place at three seawater fishponds. One was at Ria de Aveiro, a seawater lagoon with a total area of 47 km² and a freshwater income of 3–60 m³ sec⁻¹, varying with seasonal precipitation and runoff phenomena. The other two stations were located at Mondego River estuary. Mondego River has a hydrological basin of 6,670 km², and the freshwater influence at these two stations is greater than that at the first station (Fig. 1).

Environmental Conditions

Seawater was sampled every 2 wk at each site. Temperature at the different sites was measured with max-min thermometers, accurate to 1°C, that were read at the time of collection. Salinity was determined with an optical refractometer ATAGO S/Mill. To analyze particulate matter, three water samples from each site were, on each sampling date, filtered through GF/C glass fibre filters that had previously been heated to 540°C for 5 h and weighed. The filters were then dried to constant weight at 60°C, and the weights were noted. They were thereafter heated to 540°C for 6 h, allowed to cool, and weighed. The total particulate inorganic material (PIM) in the sample was obtained by subtraction of the initial weight from the final weight. The weight of the particulate organic

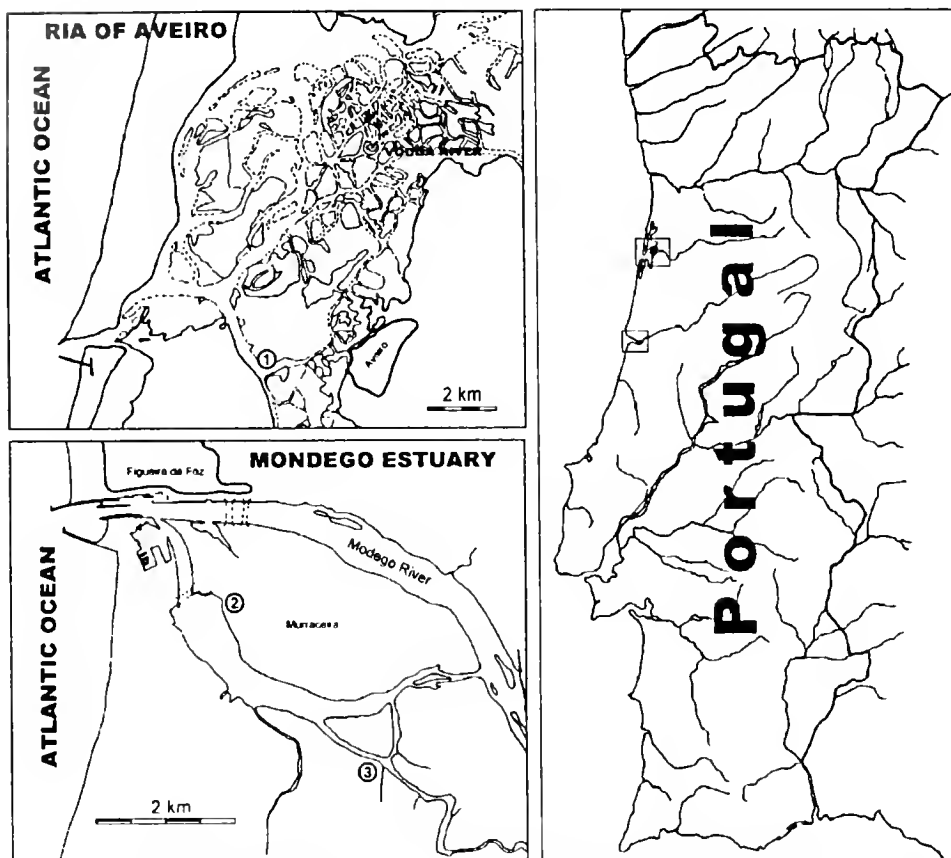


Figure 1. Location of the three stations: (1) Marinha; (2) INIP; (3) Gil.

material (POM) was calculated as the difference between the final filter weight and the weight after drying at 60°C.

Growth Experiment

Oysters were grown in pill-shaped baskets, made of rigid plastic divided into four compartments with a 1-cm mesh size. They measured 40 cm in diameter by 10 cm in height. Six trays constituted a stack, the topmost tray acted as a lid, and the bottom-most tray held a weight. Baskets were always located in the water circulation channels of the three fish farms.

C. gigas juveniles with a mean shell height of 20.1 ± 6.4 mm used in this experiment came from Marennes-Oleron (France) and were obtained from natural spatfall. About 1,000 oysters, all from the same original stock, were placed at each station. Water depth over the oysters was about 40 cm, and the water level in the different stations varied very little so that the oysters were never exposed. At every field trip, all of the baskets were agitated in the water to remove accumulated silt and feces. Every 45 days, a sample of 30 oysters was randomly selected from each station, and the mortalities were recorded.

At the laboratory, oysters were scrubbed under running tap water to remove encrusting organisms. Shell height was measured, with vernier calipers, as the distance from the end of the umbo to the ventral shell margin, and mean live weight was determined. Oysters were then opened, and tissues were excised. Wet meat weight was determined after the extracted meats were superficially

dried with absorbent tissues. Shells were rinsed with distilled water and dried for 24 h in a desiccator before weighing.

Dry meat weight was determined after oven drying at 100°C to constant weight. Samples were then ashed at 550°C in a muffle furnace, and the percentage of ash in the samples was calculated. Protein was determined by the Kjeldahl digestion of samples, and nitrogen values were multiplied by 6.25 to provide an estimate of protein. Glycogen was determined by the anthrone method described by Fraga (1956) and Strickland and Parsons (1982). Lipids were solvent extracted from samples and determined by weight after evaporation (Folch et al. 1957). Petroleum ether was used for extraction.

Length growth function was estimated with the von Bertalanffy growth model, which has a good fit for describing the growth process in molluscs (Berthome et al. 1986, Caddy 1989, Sukhotin and Maximovich 1994), $L = L_{\infty} \times (1 - e^{-K(t - t_0)})$. Condition index (CI) was calculated from the dry weights of meat and shell according to the formula $CI = \text{dry meat weight (mg)}/\text{dry shell weight (g)}$ (Walne and Mann 1975).

Statistical Analyses

Analysis of variance was used to determine any statistically significant differences in the data. Homogeneity of variance was evaluated with Levene's test, and normality with Kruskal-Wallis's test. To satisfy the assumption of normality and/or homogeneity of variance, the PIM and POM data were transformed ($\log x$). Arcsine transformation was carried out on biochemical data, which were compared as percentages. The statistical analyses

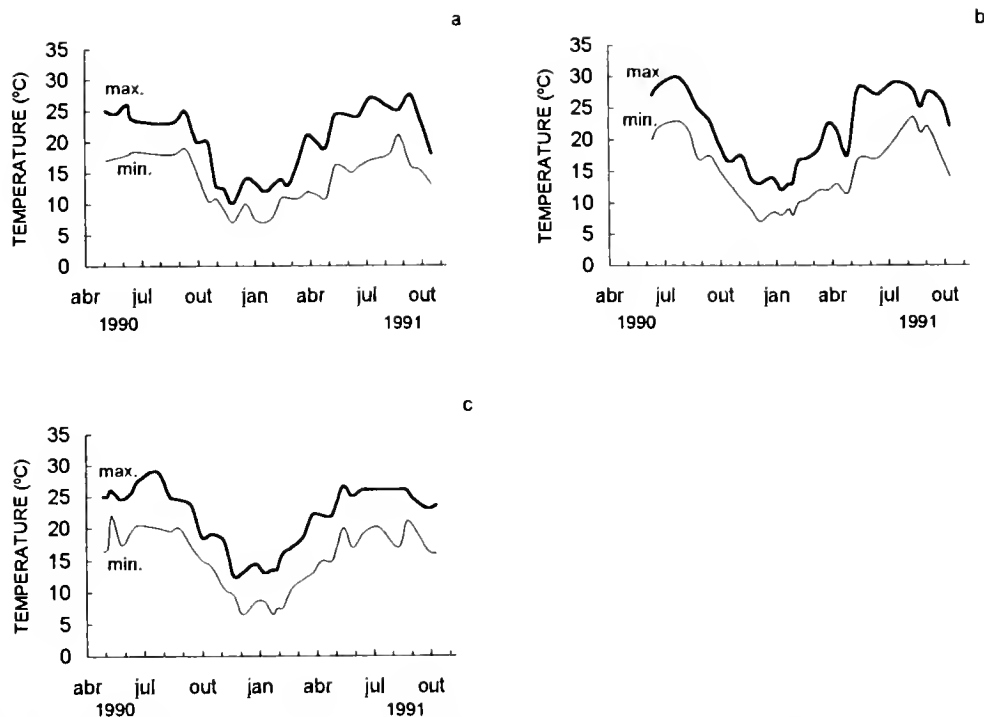


Figure 2. Seawater maximum and minimum temperatures at the three stations: (a) Marinha; (b) INIP; (c) Gil.

were carried out with the STATISTICA 4.5 (Windows 95) statistical package.

RESULTS

Environmental Conditions

Figure 2 shows seawater temperatures at the three stations from June 1990 to October 1991. Temperatures ranged from a minimum of 7°C in December 1990, at all stations, to a maximum of 30°C in July 1991 at Station INIP. The maximum value recorded at Station Marinha was 27°C, and at Station Gil, it was 29°C. The coldest periods were between December and February, and the warmest were between June and September. Mean temperatures at Stations Marinha, INIP, and Gil during the experimental period were 16.9, 18.1, and 18.5°C, respectively (number of observations = 34).

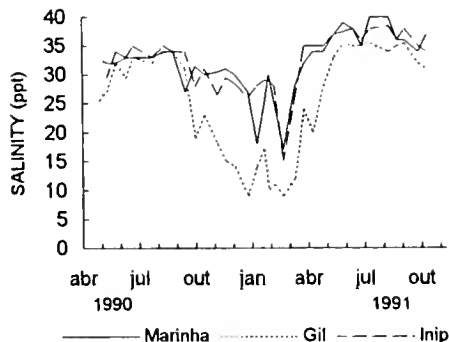


Figure 3. Seasonal variations in salinity at the three stations from May 1990 to October 1991.

Minor differences in salinity were recorded between Stations Marinha and INIP (Fig. 3). The mean salinity was 31.9 ± 5.3 , 32.3 ± 5.0 , and 25.4 ± 9.2 ppt for Marinha, INIP, and Gil, respectively. Salinity values at Station Gil, throughout the experimental period, are significantly lower ($p < 0.01$) than those at the other two stations. The larger fluctuations at Station Gil were the result of depression of salinity due to freshwater drainage from surrounding land in autumn and winter. At this station, salinity reached a minimum of 10 ppt in December and between February and March.

POM values varied within 1.5–6.7, 1.2–11.2, and 0.5–11.2 mg L⁻¹ at Marinha, INIP, and Gil, respectively (Fig. 4a). The mean POM content was 3.1 ± 1.4 , 3.7 ± 2.6 , and 3.3 ± 2.6 mg L⁻¹ at Marinha, INIP, and Gil, respectively.

PIM values varied within 4.7–23.6, 2.9–26.0, and 2.0–48.3 mg L⁻¹ at Marinha, INIP, and Gil, respectively (Fig. 4b). At Gil, extreme seasonal maxima of 48.3 and 44.3 mg L⁻¹ were observed in January and March 1991. There are no significant differences between POM and PIM levels at the different stations.

Oyster Growth

Mean shell heights (mm) for *C. gigas* at the three stations from June 1990 to October 1991 are plotted in Figure 5a. Growth in shell height was almost constant from June to October 1990. Growth in height ceased from November 1990 to March 1991. The value of parameter K of the Von Bertalanffy growth model is presented in Table 1. Station Marinha had a maximum value of $K = 0.0113$, compared with the other two stations; nevertheless, this difference is not significant.

At the end of the experimental period, oysters had a mean live weight of 53, 50, and 38 g, at Stations Marinha, INIP, and Gil, respectively (Fig. 5b). There were significant regressions of log shell weight on log shell length (Table 2). For a given shell length, shell weight is heavier at Station Marinha.

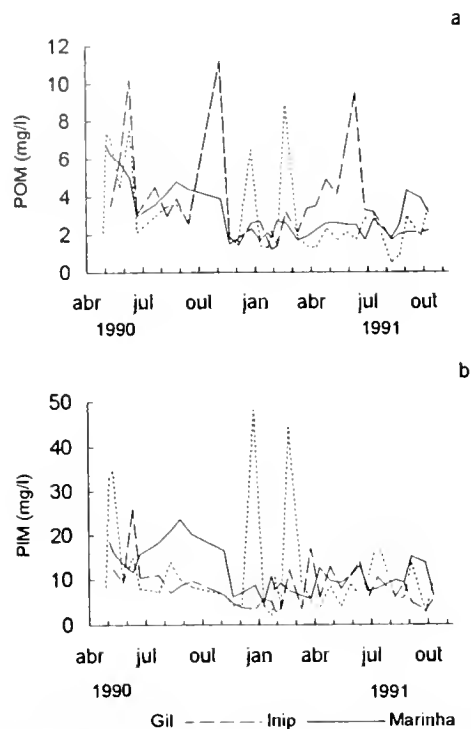


Figure 4. Seasonal variation in (a) POM and (b) PIM at the three locations throughout the experimental period.

Oysters at Station INIP reached a maximum dry tissue weight in May 1991 of 1,740 mg, whereas the maximum weights at Marinha and Gil were 1,430 and 830 mg, respectively. At Station Gil, there was not an increase in tissue weight during spring 1991, in contrast to weights at the other stations (Fig. 6a).

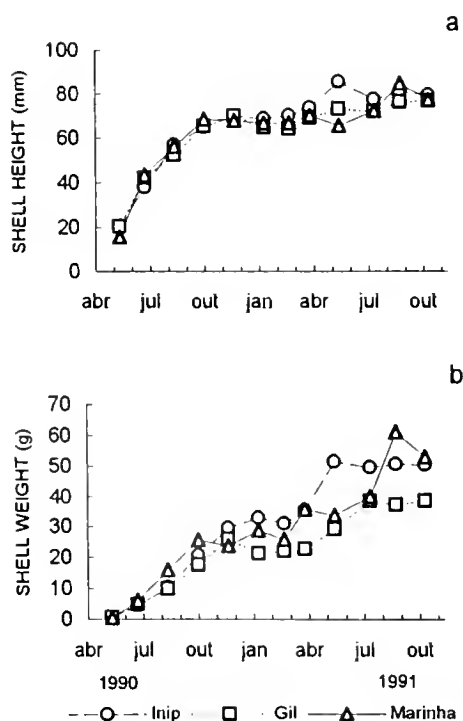


Figure 5. (a) Mean shell heights and (b) weights for *C. gigas* at the three stations from May 1990 to October 1991.

TABLE 1.

Results of parameter K from Von Bertalanffy length growth model.

Station	K	r^2
Marinha	0.0113	0.90
INIP	0.0065	0.96
Gil	0.0077	0.96

CI was higher during the spring than the rest of the year. The highest index at Station Gil was registered in March 1991, with a value of 64, whereas at the other two stations, it was in June (Fig. 6b), with values of 68 and 73 at INIP and Marinha, respectively.

Seasonal variations in ash content are similar at all of the stations, with minima values, around 9%, in March. The only exception is the maximum value of 22% in June at Station Gil (Fig. 6c). In general, meat water content is higher in the oysters collected in winter. Oysters from Station Marinha show a slight tendency to have less meat water than the oysters from the other two stations (Fig. 6d). Mean percentages of meat water content are 80.0, 81.8, and 83.5% at Marinha, INIP, and Gil, respectively.

Mean values of protein, carbohydrates, and lipid as percentages of the ash-free dry weight (%AFDW), from January to October 1991, are shown in Figure 7. At Stations Gil and Marinha, lipid percentage shows a minimum value of 2.8 and 1.4%, respectively, in April, recovering to maxima values of 10.3% in June at Station Gil and 10.7% in July at Station Marinha. Lipid values at Station INIP are more or less constant throughout the year (Fig. 7a). Carbohydrate content shows a similar pattern of variation between stations, with higher values in March at Station Marinha (26.1%) and in April at Stations Gil (35.8%) and lower values in summer and winter months (Fig. 7b). Mean carbohydrate levels are significantly higher at Station Gil than at Stations INIP ($p < 0.05$). Protein percentage shows an increase at Stations Gil and INIP from January to October 1991, whereas at Station Marinha, protein content is more constant throughout the year (Fig. 7c). Mean protein percentages are 60.8, 71.1, and 65.1% at Marinha, INIP, and Gil, respectively. Protein percentage is significantly higher, throughout the experimental period, at Station INIP than at Station Marinha ($p < 0.05$).

Mortality

Oysters from Stations INIP and Gil showed a high mortality rate. In the first station, mortality began in the middle of July 1990 and lasted until the end of the experimental period, although the highest mortality rate was observed between July and October 1990. About 50% of the oysters that were growing at this place died during the experimental period. At Station Gil, 70% of the

TABLE 2.

Allometric relationship for shell weight (shell weight = aL^b) on shell height for oysters at the three stations.

Station	a	b	r^2	n
Marinha	$7.0E^{-5}$	2.91	0.96	11
INIP	$3.5E^{-5}$	3.00	0.95	12
Gil	$4.2E^{-5}$	2.93	0.93	12

oysters died between April 15 and July 31, 1991. Mortality at Station Marinha was negligible.

Oysters from Stations INIP and Gil presented shells infested by the worm *Polydora* sp. The degree of infestation observed in most of the sampled animals was severe, classified as IV on the scale of Catherine et al. (1990), from I to IV. At Station INIP, from January 1991 forward, 90% of the sampled oysters were infested by the

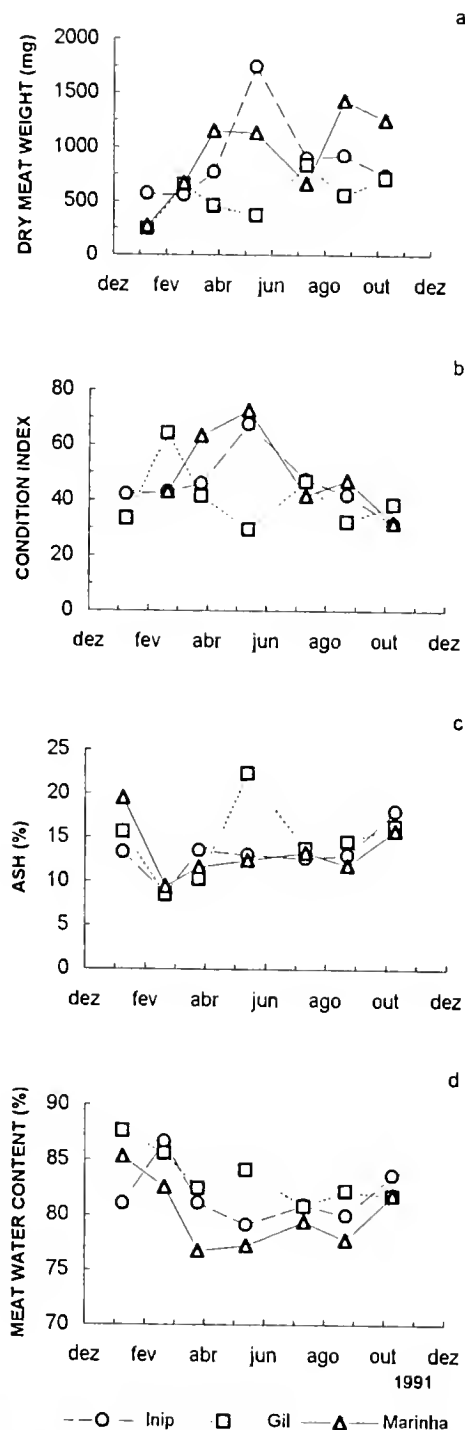


Figure 6. Mean values of (a) dry meat weight; (b) CI; (c) ash; and (d) meat water content of *C. gigas* reared at the three stations, from January to October 1991.

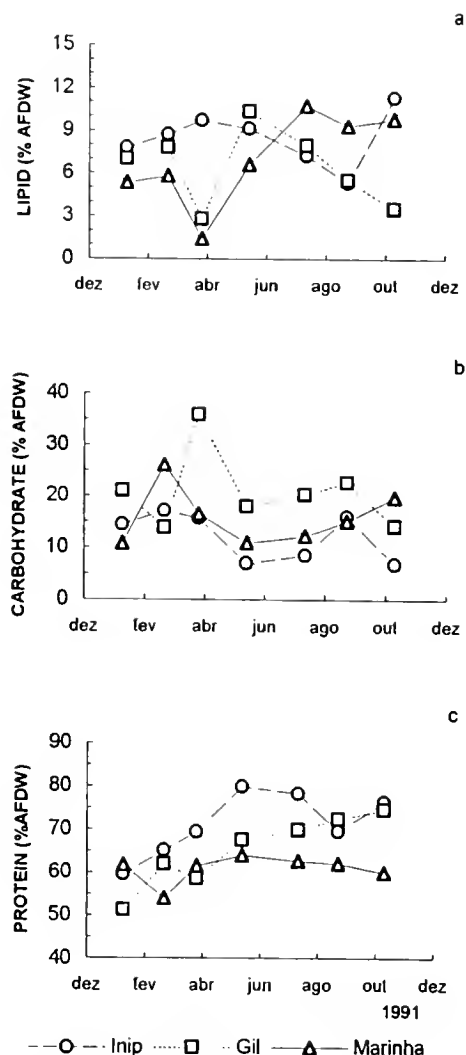


Figure 7. Seasonal variation in (a) total lipid; (b) carbohydrate; and (c) protein content of *C. gigas*, as a percentage of the AFDW, reared at the three stations, from January to October 1991.

worm, and in the last three samplings, all of the oysters had been infested. At Station Gil, the infestation percentage was much lower, with approximately 50% of the oysters infested by *Polydora* since June 1991. Before this date, the infestation was very rare.

DISCUSSION

Water circulation determines the amount of food supply available to the oysters. At both Mondego stations, water circulation is very reduced, mainly because they are built at a high level, allowing only water renovation at the higher tides. Marinha station, in addition to being a fish farm, can renovate its water daily, providing the oyster more food.

Kirby-Smith (1972) demonstrated that bay scallops (*Argopecten irradians concentricus*) grew faster in strong tidal currents because of higher rates of water exchange. The suggestion by Walne (1972) that filtration rate of *O. edulis* is directly dependent on flow rate has been criticized by Hildreth and Crisp (1976), but at flow rates below a critical level, filtration rates are reduced by filtered water being recirculated (Riisgard 1977). This fact is

normally acknowledged in the criteria that have been formulated for the selection of suitable sites for oyster cultivation. Nevertheless, King (1977), in an experiment where he cultivated *C. gigas* in a nontidal hypersaline pond, stated that this oyster species could be cultured in enclosed hypersaline areas that are nontidal. Hughes-Games (1977), also after a growth experiment, stated that *C. gigas* survived and grew well in subtropical fish ponds.

The deficient water circulation at both Mondego stations also had the inconvenience of allowing shell infestation by the worm *Polydora* sp. These worms, besides having a ubiquitous distribution, can only perforate the shells when the water current is very low or nonexistent. Oysters are affected when planktonic larvae settle onto the inner shell surface at its growing margins. The worm then constructs a U-shaped burrow, lined with mud and detritus, with two openings to the outside (Zottoli and Carrier 1974, Sato-Okoshi and Okoshi 1993). The oysters that show medium and high infestations by *Polydora* lose all of their commercial value. In addition, the bivalve secretes an organic membrane to protect itself from the worm and, in doing so, expends a great amount of energy (Kent 1979, Wargo and Ford 1993, Almeida et al. 1996b).

The infestation by *Polydora* appeared in the winter at Station INIP and only at the end of the following spring at Station Gil. Stephen (1978) observed, in *Polydora ciliata*, that when the oysters are subjected to low levels of salinity, the worms disappear from the shells. The cited observations seem to be in agreement with the fact that only at the end of spring 1991 do the oysters start to be infested by the worms, at Station Gil. The most favorable season for *Polydora* infestation is the autumn (Deltreil and Marteil, 1976). In Station Gil, at this time of the year (end of autumn 1990), there was a decrease in salinity that lasted until the following spring. Low salinity probably inhibited *Polydora* to perforate the shell, during autumn and winter. At Station INIP, where the salinity was kept high during the winter, the worms had the opportunity to perforate the shells, at the end of autumn 1990, which resulted in the formation of blisters in the internal face of the shell (Almeida et al. 1996a).

Oysters from Station Gil were exposed to low salinity (<18 ppt) for several months (November to March), which did not appear to affect the growth rate of oysters. It is well known that in stressful conditions, oysters can preferentially promote shell growth over body tissue growth (Salo and Leet 1969, Walne and Mann 1975, Shpigel and Blaylock 1991). Nevertheless, other authors refer to a deleterious effect of low salinity on bivalve growth rates (Bernard 1983, Bayne and Newell 1983, Brown and Hartwick 1988, Toro et al. 1995). Bernard (1983) determined critical salinity for the oysters to be between 8 and 12 ppt, whereas a decrease in ventilation function was observed to occur around 18 ppt. The decrease in salinity values in winter at Station Gil is due to the influence of fresh water from Pranto River, to the distance to the sea, and to the heavy rains registered that winter. These stressful conditions were probably compensated by the high POM content verified during the winter. Mean POM content registered from November 1990 to March 1991 was 4.0 mg L^{-1} at Station Gil compared with 3.0 and 2.3 mg L^{-1} at Stations INIP and Marinha, respectively. Nutrient inputs associated with freshwater discharges from nearby river systems during this period probably increased phytoplankton biomass at Station Gil. Freshwater inputs in estuarine systems promote the increase in pheopigments over chlorophyll (Héral et al. 1983). The level of pheopigments in the water is positively correlated with oyster meat production, in particular, protein and lipid

(Héral et al. 1984). Jones and Iwama (1991) reported a strong correlation of POM, an indicator of available food, with instantaneous growth rates, in agreement with the results of other investigators (Malouf and Breeze 1977, Widdows et al. 1979, Brown and Hartwick 1988). Nevertheless, although the other two stations showed an increase in dry meat weight content in spring, oysters from Station Gil showed no increase. The high mortalities observed at this station between April and July 1991 were probably related to the low condition associated with the beginning of gonad growth. Deslous-Paoli and Héral (1988) observed that, for 2-y-old oysters, at Marennes-Oleron bassin (France), the reproductive effort is responsible for 63% energy loss. Oysters sampled in May at this station showed high ash and meat water content. Meat quality is related to ash and water content and the level of storage products. Low-quality meats have high ash and water contents (Haven 1962, Shaw et al. 1967, Deslous-Paoli and Héral 1988).

The high mortality recorded at Station Gil apparently had nothing to do with *Polydora* infestation, because infested oysters started to appear in June 1991. The mortality that occurred at Station INIP in the summer of 1990 was coincident with a high temperature period. From the environmental parameters that were recorded, this was the one that showed the more extreme values, suggesting a relationship with the mortality observed. A temperature of 30°C is estimated as the upper thermal limit for *C. gigas* (Le Gall and Raillard 1988, Bougrier et al. 1995). For these species, the filtration and ingestion rates decrease with temperatures above 20°C (Le Gall and Raillard 1988), whereas oxygen consumption rates increase (Bougrier et al. 1995), in accordance with results obtained for other bivalve species (Riva and Massé 1983, Widdows 1987, Schulte 1975). Some authors (Walne and Mann 1975, Shpigel and Blaylock 1991) observed that at high temperatures, there is a decrease in meat production or catabolism. When a bivalve is environmentally stressed, the proportion of amino nitrogen to total excreted nitrogen may increase considerably above normal (Bayne 1973, Shpigel and Blaylock 1991). Nevertheless, Station Gil showed a similar temperature curve without any significant mortality, at the same time. Hughes-Games (1977) also reported higher temperatures in his experiment ($12\text{--}34^\circ\text{C}$), with almost no mortality rates.

The peak and subsequent decline in dry meat weight levels, at Stations Marinha and INIP, are probably associated with gonad growth and spawning activity, respectively. Oysters from Station Marinha seem to have two spawning seasons, one in summer and the other in autumn, with dry meat weight maximum values in May to June and again in September. Station INIP shows only one maximum, in May. Samples of oysters examined during these periods contained ripe gonads. Carbohydrate and lipid seasonal levels are in agreement with a gametogenic cycle. As is generally found in bivalves (Brown and Russel-Hunter 1978), mature oysters give priority to gonad growth and gamete production. In bivalves, gonad development may involve the metabolic conversion of glycogen to lipid (Mann 1979, Gabbott 1983, Deslous-Paoli and Héral 1988, Ruiz et al. 1992). Biochemical composition values were similar to the values found in the same species grown in other places, using the same analytical methods (Muniz et al. 1986; Arizpe, 1996).

Growth of oysters in fish farm earthen ponds is not as good as in open water sites at the same estuaries (our data), and mortality rates at both Mondego stations were too high to be compatible with a commercial oyster farm business. Station Marinha, on the other hand, gave good results concerning condition and survival of the

oysters throughout the experimental period. Growth rates in the first growing season (May to October 1990) were similar between sheltered and open sites (our data). Except for the unexplained mortality at Station INIP, we could say that fish farms, with controlled water circulation, appear to be suitable places for intermediary oyster growth.

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CONSIDERATIONS REGARDING THE POSSIBLE INTRODUCTION OF THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*) TO THE GULF OF MAINE: A REVIEW OF GLOBAL EXPERIENCE

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ABSTRACT This report was prepared in response to an interest among representatives of the Maine oyster culture industry in potentially introducing the nonendemic species *Crassostrea gigas* into the Gulf of Maine for culture purposes. The manuscript was originally written for the members of the shellfisheries subcommittee of the Maine Aquaculture Association. Topics reviewed include: the history of oyster culture in Maine, the rationale behind the interest in introduction of the Pacific oyster, a history of *C. gigas* introduction around the world, the legal aspects of nonendemic introduction, diseases associated with the Pacific oyster, methods of inhibiting reproduction of the nonendemic species, the ecological implications of introducing *C. gigas*, and production of the Pacific oyster in Maine. The article is a compilation of both published material and information contributed through personal communications with regional specialists. The authors do not assert conclusions but offer the material assembled below as a source of information to those involved in the decision-making processes concerning proposed introductions in Maine and other geographic regions.

KEY WORDS: Pacific oyster, *Crassostrea gigas*, introduction, nonendemic, aquaculture, Maine, reproduction, interaction, ecology

INTRODUCTION

This article addresses a recurring interest among representatives of the Maine aquaculture industry in the introduction of the nonendemic species *Crassostrea gigas* into the Gulf of Maine for culture purposes. What follows is an objective review of issues that are pertinent to any proposed introduction including means of circumventing associated potential problems. The relevant information is arranged categorically, and much of the material assembled here was derived from literary sources. Additional information was contributed verbally and in writing through personal communications with many individuals. No conclusions are offered. Rather, it is expected that the members of the shellfisheries subcommittee of the Maine Aquaculture Association, for whom this article was prepared, will take into account the facts presented here when drawing their own conclusions. Further, it is hoped that presentation of these data will provide the necessary facts on which reasonable and sensible management decisions will be made.

THE HISTORY OF OYSTERS IN MAINE

The history of oysters along the Maine coast may be traced back to the earliest records of the region. Points adjacent to the Gulf of Maine where shell heaps and underwater deposits serve as vestiges to the once flourishing eastern oyster beds include: the George River, the Damariscotta River, the Sheepscot River, Casco Bay, Scarborough headlands, and the Piscataqua River (Baird and Goode 1881). The largest aboriginal accumulations of oyster shells in the world (98% *Crassostrea virginica*, the remaining 2% composed of *Ensis*, *Mya*, *Mytilus*, *Mercenaria*, and *Littorina*) are located at the head of the Damariscotta River (Myers 1965). These

middens have been estimated to include 14 million m³ of shell (Castner 1950), and specimens taken from 1 foot above the pile's base have been radiocarbon dated to approximately 2,100 y before present (Russell 1979).

The northern coast of New England has not been able to support an oyster industry reliant on an endemic, wild fishery since the early 1800s because eastern oysters (*C. virginica*) have gradually become less plentiful in Maine (with the exception of limited, isolated oyster beds in the Sheepscot and Piscataqua Rivers) (Ruge 1879). The demise of *C. virginica* in Maine began during the 18th and 19th centuries when mills, built by European immigrants, released sawdust waste into many estuaries, smothering and poisoning adult animals, while effectively eliminating suitable substrate for larval settlement (Myers 1965). In addition, the waters of the Gulf of Maine have become cooler over the last few millennia (Dunham and Bray 1974). Resulting temperature regimens no longer favor the propagation and larval survival of Transatlantic species, including *Mercenaria mercenaria* and *C. virginica*, north of Cape Cod because the relative rise in sea level, permitting greater tidal mixing and less stratification, has resulted in cooler water temperatures in the Gulf of Maine (McAlice 1981).

THE HISTORY OF OYSTER CULTURE IN MAINE

Eastern Oysters

The culture of eastern oysters in Maine using intensive methods was initiated in the 1970s by a few small, pilot-commercial ventures in the warm, upper reaches of bays and estuaries in the midcoastal portion of the state. Before this era, the only documented aquaculture efforts were the extensive practices of the Wawenocks, natives to the Abenaki State and the region now known as Maine. These native people are believed to have dived in the Damariscotta River for oysters (Russell 1979) which they traded and planted in the Sheepscot and George Rivers to maintain

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continued and convenient supplies (Baird and Goode 1881). Currently, two hatcheries produce *C. virginica* seed in this region, and the five companies that grow this seed to market size in Maine are all located on the Damariscotta River.

European Oysters

Populations of *Ostrea edulis* have been successfully introduced to Maine waters in two locations. European oysters were introduced from Holland between 1949 and 1961 by the Maine Department of Sea and Shore Fisheries in cooperation with the Fish and Wildlife Service (Dow 1970). These oysters survived, spawned, and successfully set below mean low water in Boothbay Harbor and Casco Bay (Welch 1963). In the hatchery, however, where culture of *O. edulis* has been attempted in Maine for nearly 20 y, the species has not proven hearty and exhibits inconsistent and unpredictable larval survival (Clime personal communication, Mook personal communication). The wild European oyster fisheries, which even at peak production from 1983 to 1987 produced only one-half million individuals for market, have been decimated by overfishing, lack of management, and possibly the protozoan pathogen, *Bonamia ostreae*, which was recently discovered in *O. edulis* from the Damariscotta River (Friedman and Perkins 1994, Zabaleta and Barber 1996) and other sites in Maine (Barber and Davis 1994). In France, where native beds of *O. edulis* were extensive before 1979, no populations of European oysters are reported to have recovered after decimation by *B. ostreae* (Bol personal communication). Production of European oysters in France, which was 15,000 tons in 1973, had been reduced to 1,800 tons by 1991 because of disease (Heral and Deslous-Paoli 1991).

Pacific Oysters

C. gigas represents the third and final species of oyster that has been cultured in Maine waters. In April 1949, 5 bushels of Pacific oyster seed was imported from Seattle, WA, by John Glude of the Maine Bureau of Commercial Fisheries and planted in a salt pond at Blue Hill-Sedgwick below mean low tide (Dow 1970). Those animals that survived pollution by sawdust debris reached market size of 75 mm in two growing seasons. Although gonadal development and apparent maturation occurred, surviving spat were never located (Dow and Wallace 1971). *C. gigas* were not cultured again until the early 1970s, when representatives of the species were incidentally introduced to Goose Pond at Cape Rosier, Middle Salt Pond in Blue Hill, and both Seal and Long Coves on the Damariscotta River, with shipments of *O. edulis* and *C. virginica* seed from Pacific Mariculture, Inc., Pescadero, CA (Hidu personal communication). *C. gigas* were also experimentally cultured at three locations on the Damariscotta River by researchers at the Darling Marine Center during 1972 (Packie et al. 1975).

THE RATIONALE BEHIND THE CURRENT INTEREST IN INTRODUCTION OF THE PACIFIC OYSTER

Economics

The current interest in investigating the possibility of introducing the Pacific oyster to the Gulf of Maine for culture purposes originates in part from a belief by some that this species could enhance the economic development of Maine's commercial marine resources. The economies of northern Maine's rural, coastal towns, primarily located in Washington County, require stimulation via new industry (Bassano personal communication). Oyster

farming is culturally consistent with the skills and resources of those currently employed in the diminishing traditional fisheries. An oyster culture industry in Downeast Maine would provide employment to growers and processors and could be a means of circumventing the loss of communities and coastal heritage by providing an economic alternative to immigration to the cities. The governments of Denmark and Germany made the decision to introduce *C. gigas* for culture purposes after observing successful introductions of the species elsewhere in northern Europe, in an effort to provide alternative employment to coastal communities hit hard by the decline of the fishing industry (Helm personal communication). "Expanding the oyster culture industry offers the potential for improving the standard of living in rural Maine while building upon a traditional marine resource" (Beyea personal communication).

Existing Markets

Before any production of Pacific oysters in Maine, marketing and distribution of the final product should be carefully planned by targeting markets and determining their specific desires (e.g., size and form). Per capita oyster consumption has been falling steadily in the United States since before 1950 (Dunham and Bray 1974) and is continuing to decline (Kirkley personal communication). According to data compiled on food consumption by the U.S.D.A., the number of households purchasing oysters in stores, restaurants, and raw bars on a weekly basis decreased by 50% between 1978 and 1988 (Lipton personal communication). The broad market for oysters initially declined because of improved transportation, which eliminated the requirement for long shelf life of fresh products and made available alternative seafoods that required less preparation. More recently, health concerns fueled by negative press made the market for oysters even more narrow (Shapman personal communication). Contemporary markets for the commodity are very specific and can be best developed by promoting a name-brand product, cultured in the safe waters of Maine (Kirkley personal communication).

There are currently several existing markets for Maine-raised oysters that could accommodate the Pacific oyster if it were introduced. *C. gigas* production could be used to supplement the half-shell trade, currently occupied by *C. virginica*. In Virginia, Pacific oysters, imported from Washington State, are served as a raw, gourmet substitute for eastern oysters during the early spring when no eastern oysters of appropriate size are available (Castagna personal communication). Some contend that the East Coast species is superior in appearance and flavor. According to the Western Regional Aquaculture Center, the prospect for oyster culture on the West Coast is great because of the demand for Pacific oysters on the East and Gulf coasts of the United States (Chew and Toba 1991). Nearby Canada represents an additional market opportunity for Maine growers because the wholesale price of half-shell oysters in Montreal is 50% higher than that in the United States; tariffs only amount to 7.5% of the wholesale price (Ehrbar 1975). *C. gigas* presently constitutes between 30 and 40% of the oysters consumed in the United States (Smith personal communication).

The exotic species could also represent a new market product. The colorful, fluted shell of the Pacific oyster may contribute to its appeal on the half-shell. Jon Shalpack (personal communication), general manager of Legal Seafoods in Boston, MA, who purchases 30 to 40 tons of shellfish per week "would be interested in exclusive rights to such a unique shellfish product which originated from clean, cold waters."

Pacific oysters produced in Maine could also supplement the large existing market for shucked oysters, which are stewed and fried by restaurants across the United States. Maine's existing clam-shucking industry is currently underused because of lack of *Mya arenaria* abundance (Beal personal communication). *C. gigas* could be used to expand this industry (Walker personal communication).

Finally, the possibility exists that *C. gigas* production could be expanded vertically to include processed products. The United States is a net oyster importer. Imports of canned products, predominantly from Korea, more than doubled between 1970 and 1988 from 9.5 to 21 million kg (National Sea Grant 1990). Fresh smoked oysters are the only processed oyster product for which market demand is currently growing (Lipton personal communication).

Habitat Suitability

Interest in the oceanic *C. gigas* also stems from the fact that suitable habitat for *C. virginica*, an estuarine species, is very limited in Maine, because of the primarily marine coastline. Eastern oyster growth is optimal at water temperatures ranging from 20 to 30°C (Galtsoff 1964) and a salinity range of 10 to 28 ppt (Loosanoff 1965). Pacific oysters, in contrast, live and grow in water with temperatures of 4–24°C, displaying high growth rates at 15–19°C (Walne 1979) and optimal water transport at 20°C and 25–35 ppt (Quayle 1969a). As of March 1990, only 3.25 km² of Maine's 3,225 km² of coastal waters were leased for aquaculture purposes (Maine Aquaculture Innovation Center 1990). *C. gigas* could potentially be bottom cultured in many high-salinity, cold-water areas of the coast where the Transshetleran eastern oyster grows too slowly to be of commercial value.

Biological Performance

C. virginica requires three growing seasons to develop from seed to market size in the upper reaches of the Damariscotta River. During this comparatively long growth cycle, approximately 50% of the mariculture crop is lost to mortality and predation (Scully personal communication). Culture of a faster growing species could result in an increase in survival, which would reduce investment time and labor costs.

Pacific oyster seed, averaging 7 mm in length, was grown in floating trays on the Damariscotta River between July and October 1972. Although this was a comparatively poor growth year for oysters, the animals grew to mean lengths ranging from 53 to 70 mm, with a relatively small size differential between warm- and cool-water sites (Packie et al. 1975). *C. gigas* were observed pumping water at 2°C and grew through a wire mesh of overwintering tray between November and April (Chapman personal communication). "*C. gigas* have quite a high filtration even when temperature is at 5°C. *C. gigas* is more tolerant of low temperatures, and when the level of food is high enough, winter growth occurs" (Heral and Deslous-Paoli 1991).

Water temperatures ranged from -1.8 to 25°C and salinities ranged from 20 to 32 ppt at all Maine locations where Pacific oysters were grown during the 1970s. The *C. gigas* seed, received as a contaminant species in shipments of American and European oyster seed, reached market size in a maximum of two growing seasons (Mant personal communication, Richmond personal communication, Shalfont personal communication). *C. gigas* represents a very robust species that is apparently not greatly affected

by disease (Pauley et al. 1988). Although no histological analyses were made, no disease presence was observed macroscopically in any Pacific oysters cultured in Maine (Chapman personal communication, Dow and Wallace 1971, Hidu personal communication, Mant personal communication, Richmond personal communication, Shalfont personal communication).

INTRODUCTIONS OF THE PACIFIC OYSTER AROUND THE WORLD

The Pacific oyster is now established on all major coasts of the Northern Hemisphere, with the exception of the Atlantic Coast of North America, making the species the most ubiquitous oyster in the world; apparently, it can adapt to a wide range of environmental and hydrographic conditions. Harvest of *C. gigas* represents 80% of the total world production of edible oysters (Ayres 1991, Holliday and Nell 1987). The primary stimuli for the introduction of nonendemic species include economic pressures in the presence of diminishing wild fisheries resources, destruction of a fishery because of disease, and the original nonexistence of a native fishery (Mann 1979). Regarding the introduction of *C. gigas* to the mid-Atlantic region of the East Coast, European oysters have provided the basis for an important oyster fishery. The pages that follow contain characterizations of the individual introductions of *C. gigas* to several nations. In addition to a historical perspective, each description emphasizes culture methods, ecological implications, economic information, and marketing techniques that may be relevant to the proposed introduction to Maine.

Australia

Hundreds of cases containing *C. gigas* spat from Hiroshima, Kumamoto, and Miyagi, Japan, were shipped and flown to Western Australia, Southern Australia, and Tasmania between 1947 and 1952 by the Commonwealth Scientific and Industrial Research Organization, a federal government agency (Ayres 1991). Although no Pacific oysters survived in the west, these introductions marked the establishment of an oyster industry where none had previously existed in the state of South Australia and on the island of Tasmania (Thomson 1952). In Tasmania, successful spawning and recruitment occurred during the late 1950s (Thomson 1959). During the 1960s, spat was collected by members of the industry in the Tasma River, northern Tasmania, for distribution around the island as well as to South Australia (Dix 1991). Erratic spatfalls inspired the construction of a pilot-scale, commercial, Pacific oyster hatchery by the Tasmanian government and prospective farmers in 1977 (Ayres 1991). The growers established their own hatchery in 1980, and two additional facilities followed in 1985 (Dix 1991). The present industry is totally reliant on hatchery-produced seed from Tasmania (Ayres 1991).

The majority of Pacific oyster production in Australia today takes place in Tasmania, with limited growout on several leases in South Australia. Cultchless seed, grown in nursery upwelling systems to 3–4 mm, is transferred to floating upwellers and grown to 10–15 mm (Dix 1991). Growth to harvest size occurs on intertidal racks or longlines on which *C. gigas* become marketable in approximately 18 mo (Chew 1990). Their short survival time out of water dictates that the oysters be refrigerated soon after harvest (Ayres 1991, Pollard and Hutchings 1990). Pacific oysters are most frequently supplied "opened on the halfshell," fresh or frozen to Australian restaurants where they are consumed raw (Dix 1991). In 1987, the combined *C. gigas* production of South Aus-

tralia and Tasmania was 48 million oysters valued at \$10 million U.S. (Pollard and Hutchings 1990). Despite the imprudent nature of initial introductions from Japan, there exists no recorded incidence of disease on Tasmanian, Pacific oyster farms, where the low mortality rates that do occur result from predation by flatworms and fish (Dix 1991).

The introduction has not, however, been entirely without controversy. In the state of New South Wales, the Pacific oyster is considered undesirable by many who view *C. gigas* as a potentially serious ecological, social, and regulatory problem because its presence threatens a century-old industry based on the indigenous Sydney rock oyster (*Saccostrea commercialis*). In 1985, Pacific oyster spat began setting on commercial rock oyster leases in Port Stephens, 160 km north of Sydney, in locations with water temperatures ranging from 13 to 27°C and salinities of 10–35 ppt. Evidence suggests the deliberate introduction of Tasmanian spat between 1982 and 1983. Subsequent movement of Port Stephens stock has led to *C. gigas* establishment in many other estuaries on the East Coast of Australia, sometimes reaching nuisance proportions because of excessive spatfalls (Ayles 1991). Because Pacific oysters grow faster than Sydney rock oysters, they interfere with stick culture of the native oyster by outgrowing them. In 1985, the New South Wales Agriculture and Fisheries Department declared the Pacific oyster a noxious fish, making culture and presence of the oyster on leases a legal offense. In hopes of limiting the distribution of *C. gigas* and curtailing the problem of juvenile settlement on existing crops, which results in increased culling operations for spat removal, all Pacific oysters on a lease must be destroyed before any Sydney rock oysters may be removed (Pollard and Hutchings 1990).

New Zealand

C. gigas spat of Miyagi prefecture were first discovered on the North Island of New Zealand in 1970 (Andrews 1980); however, old shell specimens have been dated back as far as 1958 (Pollard and Hutchings 1990). The four proposed sources of this accidental introduction include: spawn from Pacific oysters clutched on the hulls of Japanese and Korean squid vessels, discarded individuals that subsequently spawned, larvae released in ship ballast discharge, and larval drift across the Tasman Sea from Australia (Bourne 1979, Parameswar 1991). *C. gigas* has made rapid gains, establishing itself alongside the native *S. commercialis* in most rocky, intertidal inlets and mangrove areas, where water temperatures range from 14 to 22°C and salinities vary between 16 and 35.5 ppt (Dinamani 1991). Although the two animals coexist, the Pacific oyster has become the farmer's species of choice over the rock oyster because, even though both have a similar market value, *C. gigas* reaches harvest size in 15–18 mo (Pollard and Hutchings 1990) as opposed to *S. commercialis*, which requires 2–3 y grow-out time (Parameswar 1991). *C. gigas* has recently been found among the valuable green mussel beds of Marlborough Sound, New Zealand (Chew 1990).

C. gigas cultivation has represented a significant business in New Zealand. The industry employed 150 individuals in 1990. *C. gigas* were cultured on 350 ha of intertidal shoreline where wild, collected spat were grown on racks. Two thousand tons of Pacific oysters valued between \$2.7 million and \$3.2 million U.S. were produced in 1985 (Dinamani 1991).

Researchers at the Food and Technology Department of Massey University, New Zealand, found that *C. gigas* require prompt

chilling in order to prevent bacterial growth that resulted in deterioration of organoleptic qualities including flavor, odor, and texture. Pacific oysters maintained at an ambient temperature of approximately 11°C could be held for only 6 days; at 2–3°C, shelf life extended to 13 days, whereas oysters kept at 0°C were stored for at least 17 days with no quality depreciation. Local growers chilled *C. gigas* in a freshwater-ice slurry at the time of harvest and stored the oysters in cardboard cartons at 0°C on land (Boyd et al. 1980).

France

The introduction of *C. gigas* to France resulted in the establishment of a new industry while contributing to the decimation of oyster fisheries already in existence. The relatively small importation of 900 kg of Pacific oyster seed from Japan by French oyster farmers in March 1966 was followed by the outbreak of a viral gill disease known as gill necrosis virus (GNV), which plagued local beds of *Crassostrea angulata* (Andrews 1979). The malady had been previously diagnosed and described by Ferreira and Dias (1973) in Portugal as causing the gills of *C. angulata* to become notched with separation of filaments and discoloration occurring as tissues became abscessed and necrotic. Rapid spread of the gill disease resulted in a French government embargo on further importations of *C. gigas* (Andrews 1980). Between 1970 and 1972, a second syndrome, hemocytic infection virus (HIV), caused by another iridovirus and characterized by invasion of the connective tissue by blood cells and an increase in the number of brown cells, resulted in the complete disappearance of Portuguese oysters from French waters (Goulletquer and Heral 1991). According to Henri Grizel, IFREMER, France, both viruses were present in France before any *C. gigas* introductions. Because no attempt was made to isolate the viruses at that time, the truth will remain uncertain (Maurin and LeDantec 1979).

The elimination of *C. angulata* from France and the coincident termination of an industry that produced 65,000 tons of oysters annually (Grizel and Heral 1991) resulted in an official government decision to import *C. gigas* in commercial quantities to the West Coast for culture purposes (Andrews 1980). Between 1971 and 1975, 562 tons of mature Pacific oysters was introduced from British Columbia, while 10,015 tons of spat was imported from Japan between 1971 and 1977 (Grizel 1988). After these extensive introductions, two haplosporidians were found in the endemic *O. edulis* including *Marteilia refringens* (Aber disease), which inhabits the digestive tract, and *Minchinia armoricana*, a protozoan similar to *M. castalis*, a pathogen of *C. virginica* (Andrews 1979). Although further imports of *C. gigas* were banned by the French government in 1982 after the discovery of these haplosporidia in supposedly disease-free Japanese seed, Pacific oysters reproduced so prolifically on the southwest coast of France that further imports were not necessary to sustain the industry (Mann 1983). The exotic species that accompanied *C. gigas* introductions despite inspection and immersion in freshwater include the onidarian *Aiptasia pulchella*, the cirripeds *Balanus amphitrite* and *Balanus albicostatus*, and the macroalgae *Laminaria japonica* and *Undaria pinnatifida* (Goulletquer and Heral 1991).

Prodigious Pacific oyster settlement has resulted in colonization of all sites formerly occupied by *C. angulata* and some areas including Arcachon, Brittany, and Southern Normandy, where Portuguese oysters did not exist. Spawning occurred where both water temperature (>22°C) and salinity (34–35 ppt) were high

(Maurin and LeDantec 1979). Commercial oyster landings by French farmers in 1990 were recorded at 150,000 metric tons, valued at \$210 million U.S., of which *C. gigas* accounted for 92% (Grizel and Heral 1991). The present industry employs 35,000 individuals and occupies 2,000 ha of state leasing ground. Consumer acceptance has increased with production, and 97% of the crop is sold domestically, primarily in the shell to be consumed on the half-shell (Heral and Deslous-Paoli 1991).

The industry is extensive in nature, and production is almost entirely dependent on natural spatfall (Goulletquer and Heral 1991). Oysters are cultured on intertidal racks, on hanging ropes, and on bottom. Bottom culture, which produced 20 tons of mature oysters for every ton of seed sown, resulted in the highest mean yields but also required the largest capital investment, primarily in dredging boats used for harvesting and to turn the oysters regularly with forks. *C. gigas* are grown below 1–3 m of water, at densities of 5 kg of oysters m⁻² of leasing ground for "pregrowing" and 7 kg oysters m⁻² of bottom space for "maturing phase" (Heral and Deslous-Paoli 1991). The gradual reduction in growth rate of Pacific oysters since 1972, when a market size of 70 g was reached in 18–20 mo, has been attributed to overcrowding via intensive spatfalls, resulting in a growth period of 2–5 y from seed to harvest (Maurin and LeDantec 1979). Oyster overstocking has also induced sedimentation of vast quantities of biodeposits, causing deterioration of shellfish grounds (Goulletquer and Heral 1991).

The Netherlands

O. edulis has been cultured on lease grounds in southwestern Holland since 1875 (Bol personal communication). A disastrous flood in 1953 in the same region prompted construction of barriers between the North Sea and the two estuaries that represented the major center of oyster and mussel culture in the Netherlands. The Storm Surge Barrier in the Oosterschelde (eastern Scheldt) has caused a reduction in tidal volume of 35%, whereas the Grevelingen estuary, to the north, has been completely embayed by a dam. The absence of tidal exchange in Lake Grevelingen resulted in high water temperatures (above 20°C for several weeks almost every summer) and favorable conditions for settlement. The lake was consequently used to produce *O. edulis* seed, which were grown to market size in the Oosterschelde where high current speeds and relatively low summer temperatures were favorable for fattening (Dijkema 1988). However, since 1981, *B. ostreae* has caused high mortality in European oysters, predominantly in yearlings of approximately 50 g (Bol personal communication).

After an extreme winter in 1962–1963 damaged 95% of the Dutch, European oyster crop, Dr. P. Korringa with the assistance of Mr. J. Bol (personal communication) of the Netherlands Institute for Fishery Investigations introduced *C. gigas* on an experimental scale to test the oyster's performance in Dutch waters. Small amounts of 10-mm *C. gigas* spat of Kumamoto and Miyagi strains were imported from Japan to the Oosterschelde and stocked in a shallow lease site. These oysters reached market size (100 g) in two growing seasons, and a decision was made to introduce *C. gigas* to the Oosterschelde on a commercial scale under the assumption that summer water temperatures would be too low for successful recruitment (Dijkema personal communication).

C. gigas has been imported to Holland regularly from France since 1964, yet not until the unusually hot summer of 1976 did natural spat recruitment occur (Mann 1983). Spawning and spatfall in subsequent, warm summers have been profuse, resulting in the

establishment of wild, reeflike oyster banks on the sandflats of the intertidal zone and on the slopes of the flood barrier dikes (Bol personal communication, Dijkema personal communication). Sustained summer water temperatures of 21–22°C have resulted in extensive expansion of Pacific oysters in the Oosterschelde (Bol personal communication), and since 1986, *C. gigas* has been found incidentally in the Westerschelde to the south and Lake Grevelingen to the north (Dijkema personal communication). No diseases are known to have been introduced with Pacific oysters (Bol personal communication).

Although *O. edulis* is marketed at four to five times the price, devastation by *Bonamia* has resulted in the predominance of *C. gigas* cultivation, which is more labor intensive (Bol personal communication). Mussel shell, used as cultch, is dredged after spatfall, broken into shell pieces, and seeded for bottom culture below the mean low-water mark in the tidal area and also in deeper plots. Water temperatures of the Oosterschelde range from –3 to 24°C, and salinities vary between 28 and 30 ppt (Dijkema personal communication). A small commercial nursery has recently raised imported French, Pacific oyster seed to 10 mm in an upwelling system and to 35 mm (5 g) in suspended trays. These animals have not survived their final growth phase on bottom because their shells, which were thin compared with those of naturally recruited seed, made these hatchery-produced oysters more vulnerable to predation by starfish (Bol personal communication). Between 700 and 1,000 tons of *C. gigas* is produced each year by the Netherlands and sold primarily to Belgium and Germany but also to France (Dijkema personal communication).

The Pacific Northwest, United States

C. gigas was first introduced to the West Coast of the United States to supplement dwindling stocks of *Ostrea lurida*, the Olympic oyster, native to Washington. The decline of this fishery has been attributed to overharvesting, poor management, disease, and adverse winter weather (Chew 1979). At its peak in 1890, harvest of wild *O. lurida* produced in excess of 130,000 bushels before its subsequent, and rapid, decline (Clark and Langmo 1970). Olympic oysters required 4 y to reach their maximum size of only 50 mm and probably could not have fulfilled the needs of the Pacific Northwest, a region encompassing vast areas suitable for extensive oyster culture (Andrews 1980). Whether or not the introduction of Pacific oysters contributed to this native's decline is a matter of debate (Beattie 1983). *C. gigas* is more resistant to some environmental stresses and diseases, possibly enabling the exotic to out-compete Olympic oysters for space (Dinamani 1981). The hypothesis that chemicals released by *C. gigas* inhibit the setting of *O. lurida* has also been proposed (Chew 1979).

Adult *C. gigas* from Japan were first imported to Puget Sound, WA, by companies (Clark and Langmo 1970) and Japanese-American residents (Kincaid 1951) in 1902 after several attempts to introduce *C. virginica* between 1900 and 1902 proved unsuccessful (Chew 1979). Although these Pacific oysters suffered high mortality during transport, spat culched to their shells survived well (Chew 1990). Large-scale culture of *C. gigas* in Washington was consequently established via imports of seed from Miyagi and Kumamoto prefectures (Chew 1979). In 1928, 40 cases were relayed to Willapa Bay, representing the first Japanese seed to be planted in U.S. waters. Willapa Bay received a subsequent shipment of 3,000 cases in 1929. Plantings in British Columbia, Oregon, and California soon followed (Chew 1987). By 1940, the

production of shucked meats from Willapa Bay exceeded 3.8 million L (Sparks and Chew 1961).

During the 1970s, shipments of Japanese seed diminished because of the increased cost of shipping and the higher price of seed itself. In addition, the presence of naturalized, spawning populations of *C. gigas* in northern Hood Canal and southern Willapa Bay (Chew 1979) provided a local seed source as a consequence of the many years of introductions (Chew 1990). The industry in Washington has become almost entirely hatchery based (Chew 1991) because commercially feasible wild sets were obtained in only 7 of 10 y (Chew 1979). Private hatcheries produced larvae of Miyagi strain, which they set and dispersed as seed or sold directly to growers, who transferred the larvae to their own setting tanks. Remote setting of eyed larvae has been very successful in Washington, where in excess of 100,000 cases of seed were produced each year by this method in the late 1980s. Oyster larvae were well suited to distribution by shipment and could be set with a high rate of success by the experienced grower (Chew 1990).

Pacific oysters cultured on bottom was the chosen method in Washington, whereas off-bottom methods using rafts, racks, and stakes were tested and rejected because of increased costs. The high tidal range of 7–20 feet on the Pacific Coast exposes large areas of intertidal ground at low tide, many of which have proved suitable for culture (Glude and Chew 1980). *C. gigas* has been grown where water temperatures ranged from 8 to 22°C and salinities normally varied between 24 and 28 ppt but occasionally dropped to 5 ppt during periods of heavy rainfall. The species survived such reduced salinities for up to 2 wk with no signs of adverse affects (Chew personal communication). Larvae were remote set on shell clutch in plastic mesh bags that were opened at planting time when shells were spread on bottom and oysters were grown, extensively, to market size (Donaldson, personal communication). Growout time to market size varied between 2 and 4 y (Chew 1979). Common predators included starfish, crabs, birds, the oyster drill *Cerastostoma inornatum*, and the flatworm *Pseudostylochus ostreophagus*, introduced with *C. gigas* (Beattie 1983). Three methods have been used for harvest of the market-sized product including: removal of oysters by hand at low tide and use of drag (bag) dredges and hydraulic (escalator) dredges, both at high tide (Glude and Chew 1980).

C. gigas accounted for 98% of Washington's oyster production, which totaled 4.5 million kg, representing a market value of \$28 million in 1991. The remaining 2% was shared by *O. lurida* and *O. edulis* (Chew personal communication). The vast majority of Pacific oysters were shucked, sorted into several size categories, and sold either fresh or frozen. The 5% that remained were marketed whole for consumption on the half-shell (Smith personal communication). *C. gigas* produced for the half-shell trade were grown on hard bottom, resulting in a milder flavor compared with those cultured on a muddy substrate (Glude and Chew 1980). Approximately 60% of the commodity has been marketed on the West Coast through the major distribution centers of Seattle, Portland, San Francisco, and Los Angeles. The remainder has been distributed across the United States and Canada (Smith personal communication).

The introduction of Japanese oysters to the Pacific Northwest of the United States has been relatively trouble-free in light of the extensive early shipments of both seed and adults. The pests transferred with these animals include the Japanese oyster drill *C. inornatum*, the turbellarian flatworm *P. ostreophagus*, and the macrophyte algae *Sargassum muticum* (Quayle 1969b). All of these

organisms have negatively affected bivalve mollusks (Chew 1990).

During the 1960s and 1970s, major *C. gigas* mortalities occurred during the later summer months, resulting in a loss of 60–80% of the oysters on some beds. Particularly affected were those animals in their second year of growth, located in areas with poor circulation and temperatures that exceeded the normal range (Chew 1991) of 10–15°C (Smith personal communication). Extensive sampling followed by histological studies revealed no disease organisms, and physiological stress associated with spawning was initially blamed for the oyster losses (Chew personal communication, Perdue et al. 1981). *C. gigas* is an extremely fecund species of bivalve in which more than 50% of the body volume may be composed of gonad during the breeding season (Quayle 1988). However, more recently, nocardiosis, caused by the actinomycete bacterium *Nocardia* and resulting in raised green and yellow nodules on the mantle before fatality, was determined to be at least partially responsible for these recurring mortalities (Chew personal communication, Mann et al. 1991).

The United Kingdom

The introduction of *C. gigas* to the waters of the United Kingdom was made in a comparatively responsible manner and may serve as a model for introductions and transfers of aquatic species elsewhere. The first shipment of 76 adults from Pendrell Sound and Seymour Inlet, British Columbia, to the Ministry of Agriculture, Fisheries and Food (M.A.F.F.), Conwy, North Wales (Edwards, personal communication), was supplied by the Pacific Biological Station's Fisheries Research Board of Nanaimo, Canada, in June 1965 (Walne and Helm 1979): 16–18°C during summer (Helm personal communication). Additional broodstock was imported to the Conway Laboratory (Mann 1983) from the source cited above in 1972 and from Oregon in 1979 (Utting and Spencer 1992). The laboratory has been attempting to overcome the problems associated with a limited gene pool by regularly introducing new stocks. Shipments have consisted primarily of Miyagi strain, but Pacific oysters of Kumamoto prefecture, reputed to be slower growing while producing deeper shells, are being evaluated for potentially improved meat content. Although *C. gigas* grow, fatten, and undergo gonad development in British waters, natural recruitment is very limited because of low temperatures (Walne and Helm 1979).

The hatcheries of Great Britain have minimized the risk of introducing unwanted, accompanying species while producing Pacific oyster seed, the natural recruitment of which has been limited to sheltered bodies of water on England's southern coast. "No alien pathogens or parasites appear to have been associated with the introduction in contrast with the situation in France where seed and adults of foreign origin were directly relayed in coastal waters without prior quarantine" (Helm personal communication). During exceptionally warm summers, including those of 1989 through 1991, small numbers of naturally recruited spat occurred in shallow, embayed areas (Helm unpublished data; Utting and Spencer, 1992) like Emsworth Harbor, where water temperatures reached 23–24°C (Helm personal communication). These limited spatfalls have caused some concern regarding ecological implications (Edwards personal communication). The situation demands particularly close attention in an era of global warming (Helm personal communication).

Stringent legislation has significantly contributed to the pre-

cautionary nature of the introduction of *C. gigas* to Britain. The Molluscan Shellfish (Control of Deposit) Order of 1965, strengthened in 1974 and further amended in 1983, has prohibited the deposit, in any waters adjacent to England and Wales, of molluscan shellfish without a license granted by the M.A.F.F. (Helm unpubl.). Introduction of nonindigenous species for evaluation of culture potential has been permitted only through the quarantine facilities of the M.A.F.F. Fisheries Laboratory, Conwy (Utting and Spencer 1992).

The procedure followed by the M.A.F.F. Laboratory for introduction of non-native species has been quite involved. Imported broodstock were thoroughly cleaned and held in quarantine tanks, the effluent of which was collected in large-volume, outdoor, concrete tanks where it was sterilized by adding powdered sodium hypochlorite at a rate yielding 100 ppm free-chlorine. The treated water was held for a minimum of 24 h before being discharged into the sea (Spencer et al. 1977). Subsequent to spawning, the parent stock were destroyed by boiling and were buried on land (Utting and Spencer 1992). The Conwy Lab held F_1 juveniles in quarantine for 8 mo, during which time 200 animals were randomly sampled on four dates for histopathological examination. In the absence of adverse findings, the progeny were transferred to open waters for test culture by the M.A.F.F. A final sample was examined to reconfirm the population's health 4 mo later.

No shellfish were released for commercial culture until the M.A.F.F. staff was satisfied that the species had local culture potential and presented little or no risk of negatively affecting the environment (Helm unpubl.). Samples of commercially cultivated species have been periodically checked for diseases and parasites by British government staff. The adoption of these rigorous procedures has resulted in the production of only healthy *C. gigas* seed, which has been grown out within the United Kingdom and also distributed to Denmark and Germany (Helm personal communication).

The British *C. gigas* industry has been based on production by two hatcheries: Seasalter Shellfish in Kent and Guernsey Sea Farms in the Channel Islands (Helm personal communication), which have sold seed ranging in size from 2 to 20 mm (Spencer 1990). Seed were transferred from the hatchery to land-based or floating, upwelling, nursery systems and grown to 3–4 mm (0.01 g). Pacific oysters required some form of protection from wave action, siltation, and predation by crabs, whelks, and starfish until they reached a refuge size of 45 mm (10 g), at which time they could withstand the rigors of transplantation to unprotected bottom grounds. Floating trays were used to cultivate *C. gigas* through their first year of growth. Oysters smaller than 5 mm were stocked at densities of 0.02–0.2 g/cm² or 2.5 oysters/cm², whereas animals larger than 5 mm were maintained at densities not in excess of 0.5–1.0 g/cm² (Spencer 1990).

Growout took place primarily in plastic mesh bags fastened with rubber bands to intertidal trestles of steel or timber, but bottom culture has also been used (Edwards personal communication). *C. gigas* should be completely immersed during growout because Spencer et al. (1978) found that an inverse relationship existed between percentage of time exposed to air and percentage of growth increment, with growth ceasing when animals were exposed to air for 34% of the time. Pacific oysters have been successfully overwintered in deep seawater (Helm personal communication) or underground pits (Walne 1979). Market size of 90 mm (75 g) was attained in 2–4 y, depending on water temperature (Walne and Helm 1979). Temperature varied with location and

season from 3 to 22°C, with some shallow areas experiencing 25°C. Salinities ranging from 25 to 35 ppt were common, with decreases to 15 ppt after heavy rainfalls (Edwards personal communication, Helm personal communication). Growers have expected 70% survival of first-year crops and subsequent *C. gigas* survival of 90% to market size (Spencer 1990).

The introduction of *C. gigas*, now the primary species of oyster cultured in the United Kingdom (Edwards personal communication), has resulted in a stable industry because of the excellent survival, relative ease of culture, and good marketability of the species (Helm personal communication). Commercial hatcheries produced over 100 million juvenile Pacific oysters in 1989 (Spencer 1990). Small and medium-sized growers have included individuals from all walks of life, many of whom have no background in fisheries' work (Helm personal communication). Producers have sold their oysters directly to outlets including restaurants, shellfish bars, hotels, and public houses (Spencer 1990). Helm (personal communication) called the production of approximately 1,000 tons of *C. gigas* per year "small," and Edwards (personal communication), who estimated the value of 1 year's harvest at \$2 million U.S., added "but demand is growing."

Ireland

C. gigas was introduced to Ireland in 1969 from the quarantined stocks at the M.A.F.F. Laboratory in Conwy, North Wales, where the oysters were certified disease free. No foreign organisms (i.e., disease, pests, or parasites) have appeared subsequent to the introduction. All seed grown in Ireland has been hatchery produced by Seasalter Shellfish in Kent, South England, Guernsey Sea Farms in the Channel Islands, and two major hatcheries on the West Coast of Ireland. Growout techniques have mimicked those used in the United Kingdom, and the environmental conditions of Irish waters (temperature range, 3–33°C; salinity range, 17–34 ppt) were also similar. Pacific oysters reached a market size of approximately 100 g in three summer growing seasons, yet some growth occurred during the winter in milder areas. Spawning without consequent spatfall has occurred during hot summers. Although recruitment did not occur, the spawning of oysters negatively affected their marketability. However, the previously unprecedented event of natural *C. gigas* spat settlement was observed recently in a shallow, well-enclosed bay on the northwest coast. The establishment of a breeding population of Pacific oysters in Ireland is generally considered unlikely (Minchin personal communication).

The Irish Pacific oyster industry has continued to develop slowly, with an annual production in 1991 of approximately 1,500 tons (Minchin personal communication). However, a major challenge recently facing the producers has been the development of new markets (Aquaculture Ireland 1991, Grizel and Bailly 1991, Quaestus and BIM 1991). The predominant market for Irish *C. gigas* has been the United Kingdom, where demand for other species was very limited (Aquaculture Ireland 1991). The Board lascaigh Mhara (BIM) (the Irish Seafisheries Board) recently commissioned Quaestus Ltd. to develop a market-oriented strategic perspective of the industry. Quaestus and BIM (1991) advised the Irish Pacific oyster growers to concentrate efforts on the domination of two segments of the U.K. market, including the catering business and the second-level (moderately priced) hotels and restaurants. In addition, the report advised the establishment of Irish-owned depuration, handling, and packaging facilities in England, along with a quality guarantee program to support promotional

campaigns. Such campaigns would educate caterers and consumers on the handling, storage, opening, and presentation of the shellfish, while promoting oysters as a safe product of high quality by providing literature, samples, and press coverage incorporating a slogan for Irish oysters. BIM plans to follow up this report by providing advice and financial assistance to growers toward these goals (Quaestus and BIM 1991).

THE LEGAL ASPECTS OF NONENDEMIC INTRODUCTIONS

There exist both federal and state laws pertaining to introductions. The U.S. Federal Law that addresses the introduction of nonendemic species into the United States and across state lines is contained in the Lacey Act Amendments of 1981, Public Law 97-79. This law essentially requires compliance with state legislation and permitting requirements. Section 6071 of Title 12 of the Maine Revised Statutes Annotated (M.R.S.A.) authorizes the Commissioner of the Maine Department of Marine Resources (M.D.M.R.) to issue permits for "possession, importation and introduction of organisms which will not endanger the indigenous marine life or its environment." Before granting a permit allowing the introduction of a species that has not previously been introduced under a M.D.M.R. permit, the Commissioner is required to hold a hearing.

The M.D.M.R. Regulation 24 stipulates that anyone who wishes to introduce shellfish or finfish must apply for a permit from and on forms supplied by the Commissioner. All of the East Coast of North America, south of New York state, as well as Willapa Bay, WA, and many other regions are considered quarantine zones for all species of shellfish, and all such species from these areas are presumed to carry infectious diseases, pests, or parasites unless the applicant demonstrates that the shellfish have been reared in a disease-free, closed system. Any permitted broodstock must be held in quarantine, within a hatchery, the effluent from which must be treated with chlorine at a free concentration of at least 50 ppm, at least 2 h after application before discharge. Daily records of chlorination procedures must be kept.

I.C.E.S. GUIDELINES FOR NONENDEMIC INTRODUCTIONS

The introduction of a nonendemic marine species to Maine could affect the waters of other states, as well as the Atlantic Provinces of Canada. Although neither the federal nor the state laws cited above require consultation with bordering nations or states, an international policy concerning introductions has been endorsed by member nations, including countries bordering the North Atlantic. The International Council for Exploration of the Seas (I.C.E.S.) has developed a "Code of Practice" for the introduction of marine species.

The essence of the Code may be summarized as follows: "The species proposed for introduction should be studied in its native

habitat. The study should include known diseases, pests and predators, food habits, and biotic potential. To be included would be consideration of pathological, environmental, and genetic implications of the introduction. The study should extend over several years, and the results should be examined by a committee of specialists. If a decision is made to proceed, then a brood stock should be established in quarantine in the recipient country. Only the F_1 generation should be introduced to open waters, provided that no problems emerge." (Sinderman et al. 1992).

REDUCING THE ECOLOGICAL RISKS OF INTRODUCTION OF *C. GIGAS* TO MAINE

Inhibition of Reproduction Through Geographic Location

One means of reducing the risk that a nonendemic species will reproduce and establish a resident population is to introduce the animal only in locations where, historically, environmental conditions have never been compatible with those required for procreation. *C. gigas* was reported to spawn at water temperatures ranging from 16 to 30°C and salinities of 10–30 ppt. However, Pacific oyster larval survival required sustained temperatures of 18°C or above and salinities of at least 19 ppt (Mann et al. 1991). These environmental conditions had to be maintained for at least 2 wk before the pelagic larvae completed metamorphosis and became sessile animals. This relatively high required water temperature may have been the reason that no successful *C. gigas* larval recruitment has been reported in Maine, despite numerous introductions over many years. However, in those instances where Pacific oysters were introduced incidentally with other seed varieties, the fact that *C. gigas* were reared at extremely low densities may have been responsible for inhibiting the synchronized release of gametes among males and females (Clime personal communication).

Coastal water temperature charts are produced on a weekly basis by the U.S. National Oceanic and Atmospheric Administration (N.O.A.A.). Such charts, specific to the coastline of the north-eastern United States for the months of June, July, August, and September in 1989, 1990, and 1991, were obtained and referred to in writing this report. According to these charts, temperatures in the Gulf of Maine between Belfast and Eastport never reached the critical minimum of 18°C, although water temperatures did rise to 17°C at several locations including Englishman Bay (8/21/90), Frenchman Bay (8/14/90 and 8/28/90), Penobscot Bay (7/15/89), and Pleasant Bay (9/12/89). In addition, water temperatures of shallow, protected inlets were probably higher than water temperatures in the locations documented. However, the fact that these temperatures were measured at the surface, whereas *C. gigas*, if introduced, would be cultured on bottom where cooler conditions usually prevail, should be noted.

TABLE 1.
Temperature and salinity ranges of adults of *Crassostrea* species.

Species	Temperature (°C)		Salinity (ppt)	
	Growth	Spawning	Spawning	Growth
<i>C. virginica</i>	5–34 (28–32)	18–25 (23)	>5 (12–27)	>8
<i>C. gigas</i>	3–35 (11–34)	16–34 (20–25)	10–42 (35)	10–30 (20–30)

Optimal ranges given in parentheses. From Mann et al. 1991.

Technical Methods of Inhibiting Reproduction

Triploidy

Triploidy is a genetic state produced artificially in cultured finfish and shellfish resulting in three sets of chromosomes instead of the normal two contained within the nucleus of each of the animal's cells. This odd number of chromosome sets prevents triploids from accomplishing normal meiosis, making them functionally sterile (Purdom 1983). The consequent reduced gonadal development in triploid oysters is advantageous to aquaculturists when fecundity affects survival, growth, or product quality in a negative way. Increases in mortality may be correlated with the summer spawning season when oysters, particularly the extremely fecund *C. gigas*, invest the majority of their energy budget in gamete production (Allen and Downing 1986). This energy is no longer available to support somatic growth, which is thus reduced during the summer when environmental conditions are most conducive to growth. Stored glycogen, which increases the palatability of the oyster, is replaced by gonad, ramifying throughout the somatic tissue and rendering the oysters less marketable at the time of year when demand is often highest. Allen and Downing (1991) demonstrated that both consumers and growers preferred the flavor, texture, and overall quality of firm, glycogen-rich triploids over softer, gravid diploids in blind taste tests ($p < 0.001$). The primary advantage of triploidy to the Maine eastern oyster industry appears to be summer marketability because of reduced gonadic development (Shatkin 1992) and an implicit increase in meat yield (Walker personal communication).

In this context, triploidy could be used to reduce the number of reproductively competent Pacific oysters introduced to the Gulf of Maine to a minimum. The hatchery-based oyster culture industry of Maine is suited to the production of triploids. Triploid *C. gigas* account for approximately 10% of the oysters produced in the Pacific Northwest (Woog 1991). Over 50% of the oysters being planted by Coast Seafoods, Washington, in 1993 were triploid (Donaldson personal communication). Meiosis of fertilized oyster eggs may be inhibited in the hatchery through chemical shock with the cytostatic chemical cytochalasin B (CB). Because this fungal metabolite is hydrophobic, it is dissolved in dimethyl sulfoxide (DMSO) as a carrier solution before treatment (Allen 1987).

To produce triploids, eggs are treated with CB after fertilization. Fertilized eggs were sampled continuously to monitor development microscopically, and treatment was begun when approximately 50% of the developing eggs exhibited first polar bodies (Allen and Bushek 1992, Barber et al. 1992), thus exposing most eggs to CB during the extrusion of polar body II. Fertilized eggs were exposed to concentrations of 0.5–1.0 mg CB/L for 10–20 min in filtered seawater held at a constant temperature. After treatment, eggs were screened onto an appropriate sized mesh and resuspended in 0.01–0.1% DMSO to remove residual CB; then, larvae were hatchery reared in a normal fashion (Allen et al. 1989). CB has been approved by the U.S. Food and Drug Administration for the production of triploid oysters according to the protocol established by Allen and Downing (1986).

The results of a given CB treatment—the percentage of triploidy in a batch of oysters—may be assessed by several different techniques, including flow cytometry, which has proved to be both fast and accurate (Allen et al. 1989). The cells of both hemolymph and tissue, taken from spat and adult oysters, have been used for flow cytometric assessment of ploidy level in mollusks (Allen

1983). Straight hinge larvae (48 h old) may also be assayed via flow cytometry (Allen and Bushek 1992). Such early determination of treatment success allows hatcheries to avoid wasting time and space rearing batches of oyster larvae with low yields of triploids (Beaumont and Fairbrother 1991). Flow cytometry of larvae often yields a higher initial assessment of percent triploidy than the actual proportion of polyploids among the spat (e.g., triploidy in larvae—75% percent, triploidy in spat—60%) (Allen personal communication).

Treatment of *C. gigas* eggs with CB has repeatedly yielded triploidy in spat determined to be 100% via flow cytometry (Allen et al. 1989, Allen and Downing 1986). However, the possibility remains that fertile diploid animals exist within a group of oysters assessed to be 100% triploid, yet merely escaped sampling for assay of ploidy level. Although 100% triploidy may be verified in experimental trials by assaying every individual oyster, such complete analysis is simply not realistic for commercial purposes (Allen personal communication). Commercial hatcheries in the Pacific Northwest obtain 90% triploidy on average from a given CB treatment (Donaldson personal communication).

In June 1993, after more than 2 y of controversy, an experiment using triploid *C. gigas* was initiated by the Virginia Institute of Marine Science (VIMS) in the York River, VA, with a permit from the Virginia Marine Resources Commission. Researchers intended to determine whether Pacific oysters were resistant to MSX and dermo, two parasitic diseases that have decimated the native populations of *C. virginica* (Blankenship 1994). If *C. gigas* were found to be disease resistant, perhaps information concerning how they survived could be used to increase disease resistance in native populations of American oysters.

On June 29, 1993, trays containing 200 Pacific oysters and 400 American Oysters were placed in the York River. All *C. gigas* were treated with CB at Rutgers University's Haskin Shellfish Research Laboratory to induce triploidy and presumed sterility. Before the experiments, the triploid status of each Pacific oyster used was confirmed by flow cytometric assay of blood samples at the Rutgers Laboratory. Oysters were periodically removed from the York River during the study for disease as well as ploidy analysis. In October 1993, one individual *C. gigas* tested was found to be diploid. Examination of the remaining 85 oysters revealed that 20% were mosaics, i.e., contained both triploid and diploid cells. These results suggested that the animals were in the process of reverting to diploidy. Although the water in the York River was too cold to stimulate reproduction at the time that the reversion took place, the experiment was terminated by the researchers (Blankenship 1994). VIMS "does not support the introduction of non-native species as an alternative to restoration of natural populations of *C. virginica*, or as a substitute in the public fishery" (Taylor unpublished data).

As of January 1996, VIMS researchers were seeking permission from the state of Virginia to resume experiments with foreign oysters in the Chesapeake Bay. A 4-y project has been proposed using all four strains of Pacific oysters to determine which would be best suited for the Chesapeake. The "controlled experiments" would examine growth, reproduction, and disease resistance under a variety of environmental conditions (Aquaculture News 1996).

Tetraploidy

Chromosome set manipulation technologies similar to those used to induce triploidy have recently been applied to the inves-

tigation of molluscan tetraploid production. Viable, female, tetraploid oysters would spawn diploid eggs, which when fertilized with normal sperm, would yield 100% triploid progeny. Guo (1991) has attempted to induce tetraploidy in *C. gigas* using four approaches: meiosis I blocking, polar body I blocking, cell fusion, and gynogenetic egg activation. All of these methodologies produced tetraploids; however, none of the tetraploids survived past larval stage. The inviability of the induced tetraploids could not be satisfactorily explained by defects caused by induction treatments, and the hypothesis was proposed that inviability may be caused by a cell deficiency. Further studies are necessary to determine conclusively that tetraploidy is lethal in Pacific oysters.

Although molluscan tetraploidy research is not currently ongoing, Dr. Ximing Guo (personal communication) believes that two methods of producing tetraploids, using gynogenesis (all chromosomes obtained from the mother) and triploid eggs, deserve further investigation. Gynogenetic tetraploids may be produced by inhibiting both polar bodies I and II in developing eggs. These eggs could metamorphose into tetraploid animals if fertilized with sperm that has been treated with ultraviolet light, which destroys the DNA contributed by the male gamete. Further research is required in order to perfect this ultraviolet irradiation treatment technique. Additional research should also focus on the production of tetraploids by fertilizing triploid eggs with normal, haploid sperm, followed by the blocking of polar body I extrusion.

Biotechnology

Biotechnology might offer the prospect of establishing 100% sterility among desired populations of mollusks in the future. Normally, genes are transcribed or copied from one strand of a section of double-stranded DNA as a plus or sense strand of messenger RNA. This mRNA is in turn translated into a functional protein within the organism. One possible approach to assuring molluscan sterility is manipulation of the genetic material that codes for a particular protein. Through gene splicing, a piece of DNA could be inserted in the reverse orientation, which would be transcribed as antisense RNA. This minus strand would be complementary to the messenger but would not code for any gene product or protein. The antisense would form a complementary complex with the plus strand of mRNA, preventing translation of the messenger into a protein (Shatkin personal communication). This technique would be particularly applicable in the case of protein hormones required for gametogenesis (Allen personal communication).

Hormones responsible for the stimulation of gametogenesis and the genes that code for these neuropeptides in *Mollusca* are currently under investigation. Genes encoding for egg-laying hormones and the neurons that control egg laying have been identified in gastropod mollusks among both the *Aplysiidae* and the *Lymnaeidae* (Van Minnen et al. 1992). The egg-laying hormone gene is expressed in the neuroendocrine bag cells of the central nervous system in the marine mollusc *Aplysia*. Egg laying is induced and coordinated in this snail by peptide products of the egg-laying hormone (Painter et al. 1989). The neuroendocrine caudodorsal cells of the freshwater snail *Lymnaea stagnalis* control egg laying and associated behaviors (Jansen et al. 1985) by releasing at least nine neuropeptides encoded by a small multigene (Van Minnen et al. 1989), including the ovulation hormone (Schmidt and Roubos 1989). When this pond snail is parasitized by *Trichobiarzia ocellata*, a peptidergic factor called schistosomin (Hordijk et al. 1991b), released from the central nervous system, counteracts the bioac-

tivity of a number of gonadotropic hormones causing inhibition of the reproduction activities in infected snails (Hordijk et al. 1991a). In the bivalve *M. edulis*, progesterone levels have been correlated with regulation of gametogenesis in both males and females (Reis-Henriques and Coimbra 1990).

The possibility exists that an aquaculture industry could use gene-splicing technology in the future to produce sterile shellfish. A gene that codes for a specific protein hormone necessary for gametogenesis would be identified and inserted in a reverse orientation into the genome of the broodstock. In addition, the normal gene signal sequence or promoter for gene expression would be replaced with a promoter inducible by a particular compound such as a simple sugar. In the absence of this inducer or sugar, the reverse gene would be silent and the animals would reproduce. However, after treatment with the inducer, the minus strand would be made and prevent production of the necessary hormone, resulting in sterility (Shatkin, personal communication). Thus, the broodstock would be fertile, whereas the progeny, after treatment, would be 100% sterile.

RATIONALE AGAINST INTRODUCTION OF *C. GIGAS* TO MAINE

The Ecological Implications of Introducing the Pacific Oyster

The culture of exclusively putative triploid Pacific oysters, restricted to Maine waters that have historically never reached critical minimum temperatures required for spawning and successful recruitment, is not a guarantee that successful reproduction would not occur. If spawning and successful recruitment were to occur, the establishment of a resident population of Pacific oysters in the Gulf of Maine could potentially result in serious effects to marine ecology and established fisheries on the coast of Maine and ultimately elsewhere. In a worse-case scenario, Pacific oysters could find a niche in hard-bottom subtidal and rocky intertidal areas and establish reefs, displacing habitat and disrupting endemic ecology in these zones. *C. gigas* could also outcompete *C. virginica*, *M. arenaria*, and *M. edulis* for space, building reefs where these native species existed and resulting in depletion of available planktonic food for consumption by these commercially important filter feeders. Finally, Pacific oyster spat could settle on and foul the shells of eastern oysters, soft-shelled clams, blue mussels, and European oysters, reducing the market value of these crops.

Diseases Associated With the Pacific Oyster

Mann et al. (1991) have provided a complete description of organisms associated with the Pacific oyster that represent actual or potential agents of disease in bivalve mollusks. The researchers summarized their characterizations with the following: "quarantine of broodstock in a hatchery and the use of first generation offspring for any field studies, that is compliance with I.C.E.S. guidelines for introduction of non-native organisms, will prevent introduction of all disease agents listed above except viruses, bacteria and the ovarian parasite *Marteilioides chunmuensis*, which is not known to cause mortality." The viruses and bacteria that may serve as vertical vectors of disease transfer are briefly described below, along with any reported methods of diagnosis.

Viral Diseases

Viral diseases reported from *C. gigas* include oyster velar virus disease (OVVD), HIV, and GNV. OVVD has occurred in Willapa

Bay and Puget Sound in Washington, affecting Pacific oyster larvae greater than 150 μ m in shell height (Elston and Wilkinson 1985) and resulting in hatchery mortalities (Leibovitz et al. 1978). The virus has developed in the cytoplasm of epithelial cells of the velum, causing lesions (Comps 1988). Light or electron microscopic observation revealed detached, necrotic velum and copious mucus being regurgitated. General necrosis of the velum and mantle preceded necrosis of other soft tissues (Johnson 1984). Both HIV and GNV were implicated in the mass mortalities of *C. angulata* in France during the 1970s, discussed earlier in this report. GNV caused ulceration of the gills, resulting in inflammation, whereas HIV induced cytoplasmic lesions in the hemocytes and injured interstitial tissues (Comps 1988). No techniques for diagnosing these viruses have been established, but the development of cell cultures could allow for their isolation and provide large quantities of virus for the determination of immunological parameters (Ford personal communication).

Bacterial Diseases

The reported bacterial diseases associated with Pacific oysters were bacillary necrosis, Pacific oyster nocardiosis, and rickettsiae. Bacillary necrosis was caused by opportunistic pathogens known as vibrios, which were free living, requiring conditions favorable to their proliferation in order to cause vibriosis in both larval and juvenile mollusks (Elston 1984). These bacteria are naturally present in seawater, so no danger of introduction exists (Mann unpubl.). Signs of bacillary necrosis in larvae including reduction in motility, extension of foot or velum, and swarming have been observed macroscopically (Lauckner 1983). Staining with trypan blue revealed detachment of mantle epithelial cells (Elston et al. 1982). Affected juvenile oysters displayed liquefaction of the ligament and growth of bacteria into the mantle, when examined histopathologically. Disease presence in juvenile cultures was also recognized by a reduction in growth rate and a loss of coloration (Elston 1984).

Nocardiosis, characterized earlier in this report, has resulted in gaping or weak shell closure in affected animals. The mantle was slightly discolored or contained yellow, green, or brown raised nodules. Characteristic histopathological changes included an infiltration of hemocytes, surrounding aggregates of Gram-positive bacteria of the genus *Nocardia* (Friedman et al. 1991). Rickettsiae have been identified as obligate intracellular parasites found in the cytoplasm of digestive diverticula epithelial cells of many bivalve mollusks (Lauckner 1983). They have not been known to cause mortality (Mann et al. 1991). This procaryotic organism has been found repeatedly in the eastern oysters, blue mussels, and soft-shelled clams of Maine and has been observed in stained histological sections as dark, irregular masses located in the epithelial cells of the digestive diverticula (Sherburne personal communication).

Potential Interaction With the Eastern Oyster

Intergeneric interactions between *C. gigas* and all of the species listed above, with the exception of *C. virginica*, have been documented in locations where Pacific oysters have been introduced. Pacific oysters grow faster than eastern oysters (Hickey 1979) and would probably outcompete *C. virginica* if the two species were to overlap geographically (Sutherland and Osman 1991). *C. gigas* would not pose a genetic threat to *C. virginica* because all attempts to produce hybrid adults have been unsuccessful. The reproductive

potential of both species may be reduced because gametes of the two species do combine to produce nonviable progeny (Allen et al. in press).

Potential Interaction With the Soft Shelled Clam

Mya arenaria represents an important wild fishery in Maine. In 1991, 1,702 commercial shellfish licenses were sold to diggers of soft-shelled clams (Lewis personal communication). In Washington, *M. arenaria* was introduced incidentally with *C. virginica* around 1900 and has established itself in Hood Canal and Puget Sound. However, despite the fact that *M. arenaria* successfully reproduces at water temperatures between 12 and 15°C (Laursen 1966), establishing itself earlier in the season than *C. gigas*, competition with *C. gigas* limits the clam to only the very softest substrates where Pacific oysters cannot survive. In areas where hard bottom coincides with water temperatures high enough for *C. gigas* larval recruitment, Pacific oysters create a "carpet" of spat, outcompeting *M. arenaria* for food and space (Bonacker personal communication).

Potential Interaction With the Blue Mussel

The annual value of the total landings of cultured and captured blue mussels in Maine, averaged over the years 1984 through 1991, was \$1.6 million (Morril personal communication). These harvests represented 86% of the mussels landed in the United States during the 7-y period (Hurst, personal communication). On the North Island of New Zealand (Dinamani 1991) and in Washington (Bonacker personal communication), *M. edulis* is considered a fouling organism by individuals who culture *C. gigas*. However, in the Oosterschelde, Holland, Pacific oysters are considered a pest by mussel growers. Fouling of *M. edulis* by *C. gigas* in the Netherlands reduces market value of the mussels and has resulted in restricted transfer of mussels to the North Sea (Dijkema personal communication).

Potential Interaction With the European Oyster

When the two species simultaneously occupy a body of water, Pacific oysters are generally not considered a competitive threat to the subtidal European oyster. *C. gigas* and *O. edulis* are cultured side by side in England (Helm personal communication) and Ireland (Minchin personal communication), where spawning of Pacific oysters is a rare event, and no interaction between the genera are reported. However, in the Netherlands, Pacific oysters threaten cultivated beds of European oysters and must be actively removed from these culture areas (Mann 1983).

Predators

The abundance of the Pacific oyster, if introduced to Maine, would probably be controlled by many of the same animals that prey on eastern oysters. Spat of *C. gigas* were reported to have softer shells than *C. virginica* by Sutherland and Osman (1991) and, consequently, could suffer higher mortality via predation. Glude (1971) reported a 40% mortality in cultured Pacific oysters in Maine as the result of predation by starfish. The species that would be likely to act as agents of biological control of *C. gigas* in Maine include the seastars *Asterias forbesi* and *Asterias vulgaris* in the intertidal zone, the green crab *Carcinus maenas* in the intertidal and subtidal zones, the rock crab *Cancer irroratus*, benthic feeding fish, and lobsters in the subtidal zone, as well as black ducks, eider ducks, and wading birds (Beal personal communication).

Unwanted Movement of Pacific Oysters Beyond the Location of Introduction

Larval Dispersal

Pelagic larvae can be carried great distances by tides and currents. *C. gigas* spat were found 20 miles north and 35 miles south of the locations of adult oysters in the Strait of Georgia, British Columbia, Canada. "Doubtless the actual distribution was somewhat greater" (Quayle 1964). To the south, Dabob Bay in northern Hood Canal represents the most prolific local source of Pacific oyster seed in Washington, yet at certain times, larvae disappear from Dabob Bay entirely, resulting in no set at all. Hydrographic study has revealed that north wind removal of surface water layers precedes replacement by colder, larvae-free water, thus moving *C. gigas* larvae outside of Dabob Bay (Westley 1956). Pacific oyster larvae may be carried up to 200 km up and down the coast from Port Stephens, Australia, at certain times (Ayres 1991).

If spawning were to occur, the distribution of any Pacific oyster larvae present in the Gulf of Maine to remote areas would be predominantly directed by large-scale surface circulation patterns during the spawning season. The eastern Maine coastal current transports waters from the Grand Manan area down the coast of Maine to the Penobscot Bay region (Townsend 1991). The vernal circulation in the top 75 m of the Gulf of Maine is a prominent, cyclonic (counter clockwise) surface gyre, moving water south along the coast toward Cape Cod, MA (Brooks 1985).

Movement by People

The distribution of *C. gigas* from a location of intended introduction could also occur via movement of fertile Pacific oysters by humans. Andrews (1979) contends that whole coastlines, not merely specific locations, states, or bodies of water, must be considered when a nonendemic marine species is introduced. The researcher asserts that transplantation by mankind will follow a successful introduction if distribution does not occur naturally by reproduction. Andrews (1980) cited instances where individuals, after observing the vigor and growth of *C. gigas* on the West Coast of the United States, redistributed the species to Delaware, Maryland, and New Jersey. These oysters did not establish resident populations, however.

Production of Pacific Oysters in Maine

The facilities and broodstock required for introducing the Pacific oyster to Maine are readily available. Three shellfish hatcheries within the state including the Beal's Island Hatchery, Marine Bio-Services, and Mook Sea Farms all have the capability to produce *C. gigas* larvae (Mook personal communication). Dr. S. K. Allen, Jr. (personal communication) is currently maintaining several generations of Pacific oysters, originating from both the Miyagi and the Hiroshima prefectures. Recently set F_4 Miyagi spat are being grown in addition to approximately 1,000 individuals of the F_3 generation. The Hiroshima stocks are composed of F spat and approximately 1,000 F_2 animals. Limited numbers of older generations of progeny and parent stocks are also being held in quarantine at the Haskin Shellfish Research Laboratory, Port Norris, NJ. The animals have been repeatedly checked histopathologically and certified disease free by Dr. Susan Ford (Allen personal communication).

A Comparison of Different *C. gigas* Breeds

The four varieties of *C. gigas* native to the Japanese Islands are Hiroshima, Hokkaido, Kumamoto, and Miyagi. Each was named in accordance with the prefecture or geographic locality from which the strain originated. The Hokkaido and Miyagi oysters evolved in the northern portion of Japan where the character of the water is cold-temperate. The Hiroshima and Kumamoto forms of Pacific oyster are subtropical in nature (Ahmed 1975). The morphology, growth characteristics, and survival of the two types of *C. gigas* currently available at the Haskin Laboratory were described by Imai and Sakai (1961). Pacific oysters of the Miyagi strain were characterized by large, slightly wavy shells with purple streaks. They were fast growing and survived best in colder waters. The Hiroshima variety possessed a deeper shell that was also darker in color and very wavy. This Pacific oyster was a slow grower and demonstrated higher survival in southern beds.

SUMMARY

Northern New England has not been able to support an oyster industry based on wild populations of the endemic *C. virginica* since the early 1800s because of the gradual decline of this species. Members of the well-established Maine aquaculture industry are interested in investigating the possibility of introducing the non-endemic *C. gigas* to the Gulf of Maine for culture purposes. This recurring interest is generated by: regional economic forces, the presence of existing markets, the biological performance of the Pacific oyster, and the availability of suitable habitat for culture of the species. *C. gigas* has been introduced to Australia, New Zealand, France, The Netherlands, The United Kingdom, Ireland, and the Pacific Northwest of the United States and is thus established on all major coasts of the northern hemisphere, making the species the most ubiquitous oyster in the world. Introduction of the Pacific oyster to the waters of the United Kingdom was made in a comparatively responsible manner, providing a good model for introductions of aquatic species elsewhere. The procedures followed include: quarantine and ultimate destruction of broodstock, sterilization of effluent, and stringent histopathological examination of juveniles. The introduction has resulted in a stable industry because of excellent survival, relative ease of culture, and good marketability of the species. Adherence to the "Code of Practice" as developed by the I.C.E.S., including study of the species in its native habitat, quarantine of broodstock, and introduction of only certified disease-free progeny prevents incidental introduction of most diseases and all pests and predators. Although critical minimum spawning temperatures have not historically been reached in the Gulf of Maine's oyster culture areas, the potential for the prolific Pacific oyster to reach nuisance proportions and to threaten indigenous species including the commercially important *M. arenaria*, *M. edulis*, and *C. virginica* should not be discounted. The number of reproductively competent Pacific oysters introduced could be reduced through induction of triploidy, while the technologies of tetraploidy and gene splicing provide possible means of producing completely sterile oyster populations in the future. *C. gigas* are successfully cultured and overwintered on bottom and should remain completely immersed during growout as the result of an inverse relationship between time exposed to air and growth. The facilities and broodstock required for introducing *C. gigas* to Maine, including three shellfish hatcheries within the state and certified disease-free broodstock in New Jersey, are readily available.

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RESPONSE OF *CRASSOSTREA VIRGINICA* TO *IN VITRO* CULTURED *PERKINSUS MARINUS*: PRELIMINARY COMPARISONS OF THREE INOCULATION METHODS

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ABSTRACT The recent development of *in vitro* culture methods for the oyster pathogen *Perkinsus marinus* (Mackin, Owen & Collier) provides a bountiful supply of axenic parasites for biological investigation. Understanding how this parasite interacts with its host, *Crassostrea virginica* (Gmelin), is of paramount importance. Here we report and discuss the results of several preliminary experiments on the response of *C. virginica* to *in vitro*-cultured *P. marinus* and the early fate of these cultured cells. In three separate experiments, doses of 10^2 – 10^7 parasites per oyster of *in vitro*-cultured parasites were used to challenge healthy oysters (mean wet tissue weight = 13.8 g) via feeding, shell cavity injection, or adductor muscle injection. After 7 wk, no oysters from the feeding trial were infected. Most oysters in the shell cavity and adductor muscle injection trials had detectable infections, but variability was high. Mean infection intensity increased with dosage, but the effect of dosage was not significant in all trials, probably because of low sample size. The cultured parasites produced infection intensities that appeared to be markedly lower than those reported for natural cells under similar experimental dosing conditions. Sixty-two percent of shell cavity and adductor muscle injections, excluding controls, produced infections with fewer than 100 parasites per oyster, the lowest dosage used in this study. In another experiment, the early fate of cultured cells was investigated for each inoculation method by sampling rejecta over 4 days after dosing. On average, 4% of fed cells, 7% of cells injected into the shell cavity, and 13% of cells injected into the adductor muscle were recovered. Eighty-two percent of discarded parasites, many in phagocytes, were found on Day 1 postinoculation. Oysters were sacrificed on Day 4 to determine total body parasite burdens. Regardless of delivery method, total recovery of parasites (discarded and total parasite burden) was low compared with the dose administered: 4% from feedings, 12% from shell cavity injections, and 21% from adductor muscle injection. Finally, transmission electron microscopy of hemocytes removed at 2, 6, and 18 h postinoculation appeared to indicate that hemocytes can digest *in vitro*-cultured parasites, possibly explaining the low recovery rates and indicating a mechanism for the apparently low pathogenicity of cultured *P. marinus*.

KEY WORDS: oyster, *Perkinsus*, disease, *in vitro* culture, dosing method

INTRODUCTION

The recent development of *in vitro* culture methods for the oyster pathogen *Perkinsus marinus* (La Peyre et al. 1993, Kleinschuster and Swink 1993, Gauthier and Vasta 1993) provides a bountiful supply of axenic cells to investigate the parasite's biology, ecology, and interaction with its host, *Crassostrea virginica*. Researchers have already capitalized on this development to identify potential parasite virulence factors (La Peyre and Faisal 1995, La Peyre et al. 1995b), determine parasite salinity tolerance (Bureson et al. 1994, O'Farrell et al. 1995), and examine genetic interactions between parasite isolates and host populations (Bushek and Allen 1996a). Experimental infection of oysters with *in vitro*-cultured *P. marinus* will undoubtedly continue as researchers strive to understand this host-parasite interaction. Identification of the most appropriate dosage and parasite delivery methods is fundamental to these investigations.

A number of laboratory studies have challenged oysters with *P. marinus* parasites. Studies using parasites purified from naturally

infected oysters have indicated that 10 to several hundred parasites are sufficient to initiate infections (Mackin 1962, Valiulis 1973, Volety and Chu 1994, Chu and Volety 1997). Results from recent studies using *in vitro*-cultured parasites seem to indicate that considerably larger doses may be required for cultured cells (La Peyre et al. 1993, Bushek and Allen, 1996a). The dose required may also depend on the route of infection. In fact, Mackin et al. (1953) demonstrated that *P. marinus* obtained from naturally infected oysters produced heavier infections faster when injected into the shell cavity than when mixed with food. To date, all studies that have challenged oysters with *in vitro*-cultured *P. marinus* have used shell cavity or adductor muscle injection procedures with doses in excess of 10^5 cells/oyster (Gauthier and Vasta 1993, La Peyre et al. 1993, Bushek and Allen 1996a). The number of cultured cells required to initiate infections for different inoculation methods remains unknown.

Here, we report results from several dose-response trials conducted with *in vitro*-cultured *P. marinus*. Three delivery methods (feeding, shell cavity injection, and injection into the adductor

muscle) were used to challenge oysters with varying dosages (10^0 – 10^7 cells/oyster) of cultured parasites. We also report our observations on the early, postchallenge fate of these parasites and make some preliminary comparisons of how it is affected by the different inoculation methods.

METHODS

Oyster Collection and Maintenance

In 1990, before any reports of *P. marinus* infections in Maine (Kleinschuster and Parent 1995, Ford 1996), 200 oysters were shipped overnight from Pemaquid Oyster Company, Waldoboro, ME, to the Haskin Shellfish Research Laboratory (HSRL), Port Norris, NJ. The oysters were quarantined in a single 200-L cylindrical tank containing filtered (1- μ m-pore-size filter) seawater (FSW) at 25 ppt and 15°C, individually labeled, and notched near the adductor muscle; 250 μ L of hemolymph was examined for *P. marinus* (Gauthier and Fisher 1990). All assays were negative. One week before experiments, each oyster was transferred to a 2-L plastic container with 1 L of FSW (1 μ m pore size) at 25 ppt. Containers were covered with a fitted lid, aerated, and placed in an 18°C water bath. The temperature was increased over the course of a week to 27°C. During experiments, temperature fluctuated from 25 to 30°C, but averaged 28°C. Oysters were fed daily with a mixture of *Isochrysis* spp. and *Chaetoceros* spp. or Diet B from Coast Seafoods, Sequim, WA (a mixture of *Skeletonema* spp. and *Thalassiosira* spp.). Composition of the diet varied with availability of live cultures, but the total food provided averaged about 2.5×10^8 cells per day per oyster. Water was changed weekly with 25°C, 25 ppt FSW (1 μ m pore size). Waste water was sterilized with household bleach for 24 h before disposal (chlorine concentration >10 ppm as measured with an ATI Water Chex chlorine test kit [PyMaH Corp., Flemington, NJ]).

Cultured Parasites

P. marinus isolate ATCC #50508 (American Type Culture Collection, Rockville, MD = LIC-1; Bushek 1994) was used for all experiments except dose response Trial 2, which used an isolate obtained from Dr. S. J. Kleinschuster of HSRL, Port Norris, NJ, (= SJK isolate). Both isolates were obtained from oysters that had been infected in Long Island Sound, CT (Bushek 1994, S. J. Kleinschuster pers. comm.). Isolate cultures were maintained at 27°C in JL-ODRP-1 medium (La Peyre et al. 1993), modified for incubation without CO₂ as described by Freshney (1987). Two- to 10-fold dilutions were performed weekly with fresh medium during routine maintenance. Before experiments, dilution of rapidly proliferating cultures from 10^7 to 10^5 cells mL⁻¹ produced a suspension of roughly uniform-sized mature trophozoites (Bushek 1994). In preparation for inoculation, trophozoites were aseptically transferred to sterile centrifuge tubes, pelleted for 5 min at 100–200 g, and resuspended in FSW (0.22 μ m pore size filter) at 25 ppt (about the same osmolality as the culture medium). Trophozoites were gently triturated with 18- and 27-G needles to separate any clusters and then counted with a hemocytometer. On the basis of the bright refractive appearance of cells examined with phase contrast microscopy (dead cells appear dark) and the continued proliferation of cells that remained in culture, viability was estimated to be >95%.

Dose Response Trials

Three separate dose response trials were conducted to determine the number of cultured parasites required to generate infections using each of three different delivery methods. In all trials, doses were assigned to oysters by lottery and oysters were arranged in the water bath according to a randomized block design. Oysters were individually maintained as described above, and survival was checked daily. The number of parasites per oyster (parasite burden) was determined as described by Bushek et al. (1994) for each oyster at the conclusion of each trial. The mean wet tissue weight of experimental oysters was 13.8 g (SE = 0.4), and there was no significant difference in wet weight of oysters among doses or trials (one-way analysis of variance [ANOVA], $p = 0.965$).

Feeding (Trial 1)

Doses of 10^3 , 10^4 , 10^5 , 10^6 , and 10^7 *P. marinus* (ATCC Isolate 50508, Passage 5) per oyster were mixed with the daily algal diet and fed to the oysters ($n = 6$ per dose). Six control oysters received unspiked algal diets. Dosage levels were chosen on the basis of findings by Mackin et al. (1953) and Mackin (1962). Their experiments with natural parasites in oyster tissue homogenates indicated that initiation of infections by feeding parasites to oysters required doses of more than 5×10^2 cells. To minimize variation among replicate oysters due to differences in feeding patterns, each oyster was fed one-fifth of its treatment dosage on each of five consecutive days. The production of feces, pseudofeces, or both, after each feeding verified that oysters had filtered the water. Parasite burdens were determined after 55 days. No statistical analyses were performed because no infections were detected (see Results).

Shell Cavity Injection (Trials 2 and 3)

Doses of 10^2 , 5×10^2 , 10^3 , 5×10^3 , and 10^4 *P. marinus* were tested in Trial 2. Dr. S. J. Kleinschuster graciously provided the *P. marinus* isolate used in this trial. This isolate was derived from Long Island Sound oysters that were infected in Long Island Sound, but may also have been exposed to *P. marinus* from Delaware Bay at the HSRL, Port Norris, NJ. The exact number of times this isolate had been passed was unknown, but it had been passed several times. In this trial, the lowest dose was based on Mackin's (1962) estimate that about 100 natural parasites were required to produce an infection. The maximum dose of 10^4 parasites was based on the observation that two injections of 2×10^5 cultured parasites produced heavy infections and mortality within a few weeks (Gauthier and Vasta 1993). A relatively low response in Trial 2 led to the testing of an additional three doses, 10^5 , 10^6 , and 10^7 per oyster, during a third trial (see below). For all shell cavity injections, parasites were introduced into the shell cavities of individual oysters ($n = 5$ per dose) by inserting a 25-G needle into the notch previously used to sample hemolymph. Five control oysters were inoculated with FSW (0.22 μ m pore size). Care was taken to avoid penetrating soft tissues during injection. Inoculated oysters were left out of water overnight to promote retention of the parasites and then were maintained as described above. Parasite burdens were determined after 47 days in Trial 2 and after 49 days in Trial 3. Body burdens were log₁₀ transformed because infection intensities spanned several orders of magnitude. Data were then analyzed for differences among dosages with one-way ANOVA, but because Trials 2 and 3 examined different dosages with different isolates (see below) and were also temporally separated, data from these trials were analyzed separately.

Adductor Muscle Injection (Trial 3)

Doses of 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , or 10^7 *P. marinus* (ATCC Isolate 50508, Passage 17) were injected into the adductor muscle of oysters ($n = 3$ per dose). The concentration of inoculum was adjusted so that each dose could be delivered in a single 150- μ L aliquot. Three control oysters received no parasites. As noted above, 15 additional oysters received shell cavity injections of 10^5 , 10^6 , or 10^7 parasites during this challenge. Oysters were maintained as described above, and parasite burdens were determined after 49 days. Data were analyzed as in Trial 2.

Early Fate of Cultured Parasites

To examine the initial fate of parasites after inoculation, four replicate oysters per inoculation method received 10^7 *in vitro*-cultured parasites that had been stained with the vital dye neutral red. Parasites (ATCC Isolate 50508, Passage 13) were stained by incubation in a 0.08% solution of neutral red in FSW (0.2 μ m pore size) for 5 min. Excess stain was eliminated by pelleting the cells at 100 g for 5 min, removing the supernate, and resuspending the cells in FSW (0.2 μ m pore size). Four replicate control oysters received no *P. marinus*. All oysters were maintained as described above. Feces, pseudofeces, and water were collected on Days 1, 2, and 4 after the challenge. Preliminary tests had verified that *P. marinus* cells retained neutral red for at least 4 days *in vitro*. Feces and pseudofeces were collected with a pasteur pipette and transferred to disposable 10-mL culture tubes. The entire liter of water in each oyster's container was then transferred to 50-mL centrifuge tubes and spun at 1,000 g for 5 min. Pellets were combined and resuspended with 5 mL of FSW (0.22 μ m pore size) in 15-mL centrifuge tubes. All samples were fixed with 2% paraformaldehyde and refrigerated until counted. On the fourth day postinoculation, oysters were shucked and weighed and total parasite burdens were determined (Bushek et al. 1994). ANOVA methods were used to detect significant patterns in these data. Details of the statistical analyses are provided with the results.

In Vivo Intracellular Observations of Parasites

The early fate of parasites phagocytosed *in vivo* was examined with transmission electron microscopy. Nine oysters were injected via the adductor muscle with 7×10^6 live cultured parasites (ATCC Isolate 50508, Passage 9). To help interpret evidence of intracellular killing of parasites, nine additional oysters received equal doses of heat-killed parasites from the same isolate. Three oysters in each group were exsanguinated at 2, 6, and 18 h post-inoculation. Hemocytes were pelleted; the cell-free supernate was discarded and then fixed at 4°C for 1 h in a solution of 2% glutaraldehyde, 0.1 M sodium cacodylate, and 0.4 M NaCl adjusted to pH 7.4. Hemocytes were then rinsed three times for 10 min each at room temperature in buffer (0.1 M cacodylate buffer and 0.4 M NaCl at pH 7.4). Secondary fixation occurred at 4°C for 1 h in a solution of 1% osmium tetroxide, 0.1 M sodium cacodylate, and 0.4 M NaCl adjusted to pH 7.4. Cells were then rinsed three times in distilled water and dehydrated in ethanol for 10–15 min each through 30, 50, 70, 90, and 100% ethanol. Specimens were infiltrated and embedded in Quetol 651 (Polysciences, Inc.), thin sectioned on diamond knives, and then stained in 2% uranyl acetate and 0.2% lead citrate. In each preparation, several hundred hemocytes were scanned for phagocytosed *P. marinus*. Similar preparations of hemolymph from naturally infected oysters were examined for comparison. Black and white micrographs were pre-

pared of hemocytes and parasites from each treatment for morphological comparisons.

RESULTS

Dose Response Trials

Parasite burdens of control oysters were negative in all three trials, and only one oyster died during the dose response experiments. The oyster that died had received an intramuscular injection of 100 *P. marinus* during the third trial and died 41 days later. Cause of death was uncertain, but *P. marinus* was unlikely because only four cells were detected from a total parasite burden assay.

No infections were detected in any oysters fed *P. marinus*, regardless of dose (data not shown). In contrast, most shell cavity and adductor muscle inoculations produced infections (Fig. 1). Mean infection intensity increased with dosage for shell cavity (Fig. 1A) and adductor muscle (Fig. 1B) injection methods, but variability was high. Regardless of dosing method, all but 6 of the 88 oysters challenged with *P. marinus* had parasite burdens lower than the number of parasites administered.

The effect of dosage was significant in Trial 2, when lower

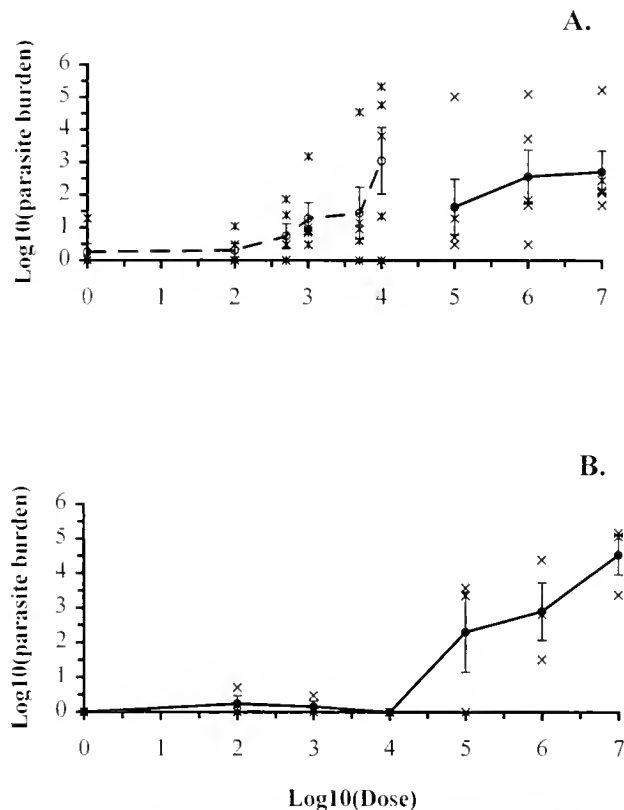


Figure 1. Dose response of *C. virginica* to *P. marinus* for two different inoculation methods. (A) Shell cavity injections. Means of five oysters are plotted with one standard error. The dashed line connecting open circles represents means for the individual oysters (asterisks) from dose response Trial 2. The solid line connecting closed circles represents means for the individual oysters ("x" symbols) from Trial 3. (B) Adductor muscle injections. Means of three oysters are plotted with one standard error. The "x" symbols represent individual oysters. The mean weight of oysters used in the experiments was 13.8 g; thus, the weight-specific doses were about one order of magnitude lower than those indicated on the x-axis.

dosages were used (dashed line in Fig. 1A, $p = 0.031$, one-way ANOVA), but not significant in Trial 3, when higher dosages were used (dashed line in Fig. 1A, $p = 0.140$, one-way ANOVA). Note that Trials 2 and 3 used different isolates. Tukey's post hoc comparison of means for Trial 2 results indicated that the 10,000 dose treatment produced infections significantly greater than the control and 100 dose treatments. Other treatments were not significantly different from each other. Examination of the raw data plotted in Figure 1A revealed a high degree of variability at all dosages. Most shell cavity injections (28 of 40) failed to produce infections of >100 cells g^{-1} wet oyster tissue. Although the high variability and low sample size obscured dose effects, a trend for mean parasite burdens to increase with dosage was evident. In Trial 2, increases in mean values were driven by increases in maximum infection intensities (Fig. 1, asterisks). In contrast, Trial 3 increases resulted from increases in the minimum infection intensities (Figure 1, "x" symbols).

The effect of dose on adductor muscle injections was highly significant ($p < 0.001$, one-way ANOVA), despite the low sample size of three oysters per dose. Dosages below 10^5 cells produced little or no infections, but higher dosages produced increasing levels of infection. This corresponds with the apparent threshold observed for shell cavity injections. Tukey's post hoc comparison of means indicated that the two highest dosages were significantly different from dosages of 10^4 parasites or less, confirming the effect of increasing dosage. For Trial 3, when dosages of 10^5 – 10^7 were contemporaneously run with the same isolate for shell cavity and adductor muscle injections, a two-way factorial ANOVA that compared the effects of inoculation methods and dose indicated no effect of inoculation method ($p = 0.926$) and no interaction between inoculation method and dose ($p = 0.389$). The effect of dose, however, remained highly significant ($p < 0.001$).

Early Fate of Cultured Parasites

Neutral red-stained parasites were found in all rejecta compartments (feces, pseudofeces, and the surrounding water) of all chal-

lenged oysters (Table 1). Variation was high, but several patterns were apparent from the data. First, a large majority of the parasites were not recovered. On average, 4% of fed cells, 7% of cells injected into the shell cavity, and 13% of cells injected into the adductor muscle were recovered during the 4 days postchallenge from rejecta compartments (Table 1). Inclusion of parasite burdens on Day 4 (Table 2) failed to account for the remaining parasites. Thus, total recovery rates remained low: 4% of fed cells, 12% of cells injected into the shell cavity, and 21% of cells injected into the adductor muscle. Second, the majority of discarded parasites (that is, those recovered from feces, pseudofeces, or surrounding water) were found on Day 1 postinoculation (69, 83, and 85% for fed, shell cavity, and adductor muscle injections, respectively; Table 1). Many of these discarded parasites were contained within hemocytes. Third, the relative abundance of parasites recovered from each compartment (feces, pseudofeces, or surrounding water) varied among delivery methods. This was most apparent on Day 1: stained parasites were most abundant in feces when *P. marinus* was fed to oysters, almost equally abundant in feces and pseudofeces after shell cavity injections, and most abundant in pseudofeces after adductor muscle injections (Table 1). Variability was, however, quite high, and a two-way ANOVA failed to detect significant effects of inoculation method ($p = 0.205$), rejecta compartment ($p = 0.407$), or their interaction ($p = 0.098$). On Days 2 and 4, stained parasites were consistently most abundant in feces for each delivery method. Cells present in the surrounding incubation water (all water was collected each day) probably represent cells from feces and pseudofeces that were not recovered with those compartments as well as cells that leaked from the mantle cavity or were expelled via diapedesis. A one-way ANOVA of Day 4 parasite burdens indicated a significant effect of inoculation method (Table 2, $p = 0.001$). Tukey's post hoc multiple comparison indicated that feeding produced significantly lower infections than shell cavity or adductor muscle injections, but the latter methods were not significantly different. Compared with feeding parasites to oysters, mean parasite burdens were 27 times heavier when

TABLE 1.
Elimination of neutral red-stained *P. marinus* cells delivered to oysters by three different methods.

Dosing Method	Day	Feces	Pseudofeces	Water	Total	% of Dose
Fed	1	197 (61)	60 (25)	47 (15)	304 (39)	= 4%
	2	38 (15)	11 (7)	21 (10)	70 (26)	
	4	39 (15)	3 (1)	22 (2)	64 (17)	
	Total	274 (47)	74 (27)	90 (24)	438 (21)	
Shell Cavity	1	185 (104)	240 (114)	130 (50)	555 (180)	= 7%
	2	11 (3)	5 (4)	14 (3)	30 (7)	
	4	47 (14)	1 (1)	34 (6)	82 (18)	
	Total	243 (117)	246 (116)	178 (51)*	667 (201)	
Adductor	1	122 (70)	840 (443)	173 (54)	1,135 (468)	= 13%
	2	45 (15)	9 (4)	31 (19)	83 (26)	
	4	53 (10)	29 (21)	24 (7)	107 (15)	
	Total	220 (65)	876 (430)	228 (58)	1,324 (441)	

The mean numbers of stained cells (thousands, \pm SE) from four replicate oysters are given for each dosing method and for each elimination compartment (feces, pseudofeces, water). The percentage of the total (10^7 per oyster) dose represented by each mean is also shown.

* After shell cavity inoculations, oysters leaked fluid, including stained *P. marinus* cells, while they were held out of water overnight. An average of 381,000 cells was found in this fluid. Including these cells, the total cells recovered from shell cavity injections is therefore 1,044,000 and the percentage of dose increases from 7 to 10%.

TABLE 2.

Mean log₁₀ parasite burdens (+/- SE), determined 4 days postchallenge, of oysters exposed to 10⁷ cultured *P. marinus* cells by three methods of delivery: F (feeding exposure), S (shell cavity injection), A (adductor muscle injection).

Method	Parasite Burden	% of Total Dose
F (n = 4)	5 (46)*	<1
S (n = 4)	136 (120)	1
A (n = 4)	821 (334)	8

* Only one of the four oysters had a significant infection of 19,000 cells.

parasites were injected into the shell cavity and 164 times heavier when parasites were injected in the adductor muscle (Table 2).

In Vivo Intracellular Observation of Parasites

Transmission electron microscopy revealed that fewer than 1% of hemocytes contained *P. marinus* 6 h postinjection. An even smaller fraction was found after 18 h. All parasites were observed within phagocytes, which typically contained fewer lysosomes than phagocytes without *P. marinus*. At 2 h postinjection, live injected parasites appeared morphologically intact within phagocytes (Fig. 2A), whereas the cell wall and membranes of heat-killed injected parasites appeared to be somewhat degraded and deteriorating (Fig. 2B). After 18 h, differences were no longer evident; that is, most parasites that had been injected alive resembled the heat-killed *P. marinus* (Fig. 2C and D). These observations imply that hemocytes can kill *in vitro*-cultured parasites. In contrast to the cultured parasites, similar preparations of hemolymph from naturally infected oysters showed a high percentage of hemocytes packed with up to 10 morphologically normal *P. marinus* cells each.

DISCUSSION

The experiments described above highlight some potential problems with the use of *in vitro*-cultured *P. marinus* in challenge experiments and provide some new insight into this host-parasite relationship. First, far greater numbers of cultured parasites were required to initiate infections compared with numbers reported in the literature for parasites obtained directly from infected oysters. Second, different dosing methods affected the host-parasite interaction, altering the rate of infection intensification and the initial response of the oyster. Finally, it appears that *C. virginica* phagocytes may be able to digest cultured *P. marinus*.

A number of studies have defined the minimum dose of natural *P. marinus* required to establish infections or cause mortality when the parasites are injected into the shell cavity. These numbers ranged from a mere 10 cells (Valiulis 1973, Chu and Volety 1997) to somewhere between 100 and 500 cells (Mackin 1962). Using Long Island Sound isolates, we found that 10⁴–10⁵ cultured parasites per adult market-sized oyster were necessary to produce a light or moderate infection in a reasonable length of time (1–2 mo). Note that total parasite burdens are reported in Figure 1 and that infections of <10⁴ cells in an adult oyster (approximately equal to

10³ cells/g) would most likely be missed with the standard tissue assay of Ray (1966, see Bushek et al. 1994) that has been used in previous studies. Hence, after similar incubation periods, the infections we detected were dramatically lighter than those reported in previous studies with natural parasites. It appears that a relatively high number of cultured *P. marinus* are required to initiate infections. This may indicate that *in vitro* culture reduces the parasite's virulence. Reduction in a parasite's virulence has been reported for other parasites and is apparently not an uncommon phenomenon (e.g., Giannini 1974, Chang and Fish 1983, Moody et al. 1990). Alternatively, the low virulence observed in this study may be specific to the Long Island Sound isolates used in this study. Bushek and Allen (1996a) demonstrated that distinct isolates can possess different levels of virulence. Such differences may explain the apparent differences in virulence between the two Long Island Sound isolates used in this study (Fig. 1). There is a need for a standard benchmark assay that can be used to measure and compare virulence among different isolates of *P. marinus*.

The comparisons of dosing methods in this study, albeit preliminary, indicate that the method used to deliver parasites to experimental oysters may play an important role in the outcome of the host-parasite relationship. Intuitively, one would expect that methods that bypass more potential barriers to infection should produce heavier infections. Results presented here generally follow this pattern. Feeding was the least invasive method used, and it failed to produce infections. Adductor muscle injection was the most invasive method used and tended to produce the heaviest infections, although shell cavity injections were not significantly lower. These findings parallel those of other investigators working with both natural and cultured parasites. Mackin et al. (1953) and Chu (1996) reported that feeding *P. marinus* parasites to oysters is relatively ineffective compared with shell cavity injection. Results from the neutral red study described in this article imply that the dose response pattern is established early in the disease process. By 4 days postchallenge, infections were already significantly higher in oysters dosed via shell cavity or intramuscular injection compared with infections in oysters dosed by feeding. Notably, the lack of clear-cut differences between the two injection methods in both the long- and short-term experiments may indicate that shell cavity injection, which is less invasive in the sense that it does not penetrate the soft tissues, is nearly as effective in establishing infections as adductor muscle injection.

Regardless of which delivery method was used, mean total parasite burdens were always lower than the inoculum. Bushek and Allen (1996b) found that parasite burdens tended to decline during the first 2 mo after oysters had been dosed via shell cavity injection with 5 × 10⁵ cultured cells g⁻¹ wet weight. Our neutral red and electron microscopy studies may shed some light on these observations. Four days after delivery of parasites, only 4% of those fed to oysters, 12% of those injected into the shell cavity, and 21% of those injected into the adductor muscle were recovered from tissues and rejecta combined. Evidently, a large majority of the parasites were not infective, were rapidly destroyed by the oysters, or were not detected by the assay. Electron micrographs indicated that cultured cells may be digested by phagocytes within hours of injection, suggesting that *in vivo* phagocytosis and intracellular killing are functional defense mechanisms against cultured parasites. La Peyre et al. (1995b) found that natural *P. marinus* parasites were engulfed and could be digested intracellularly under *in vitro* conditions. We did not examine a comparable time course

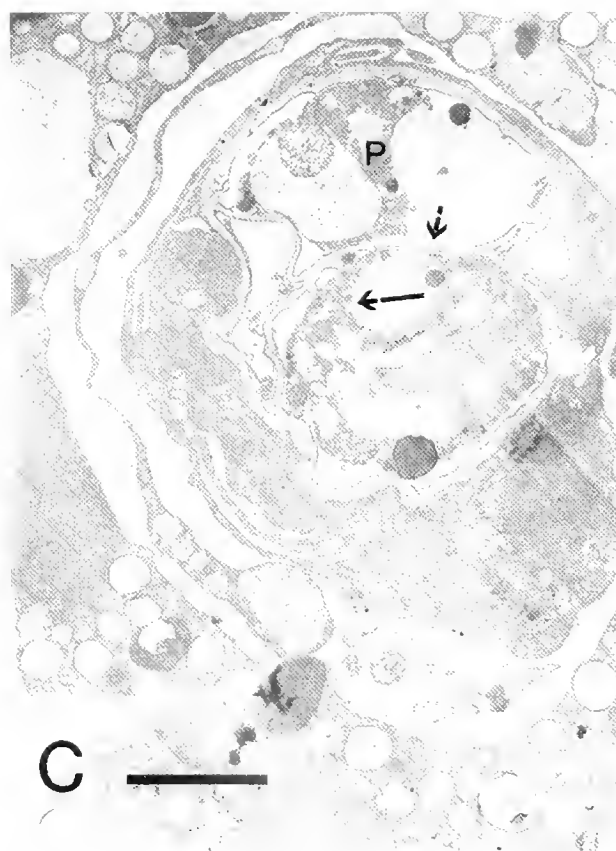
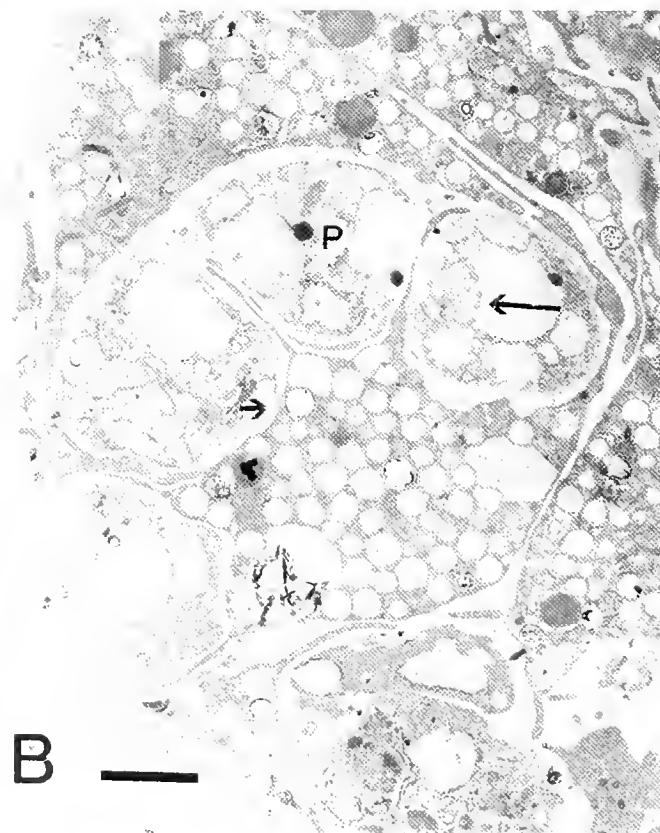
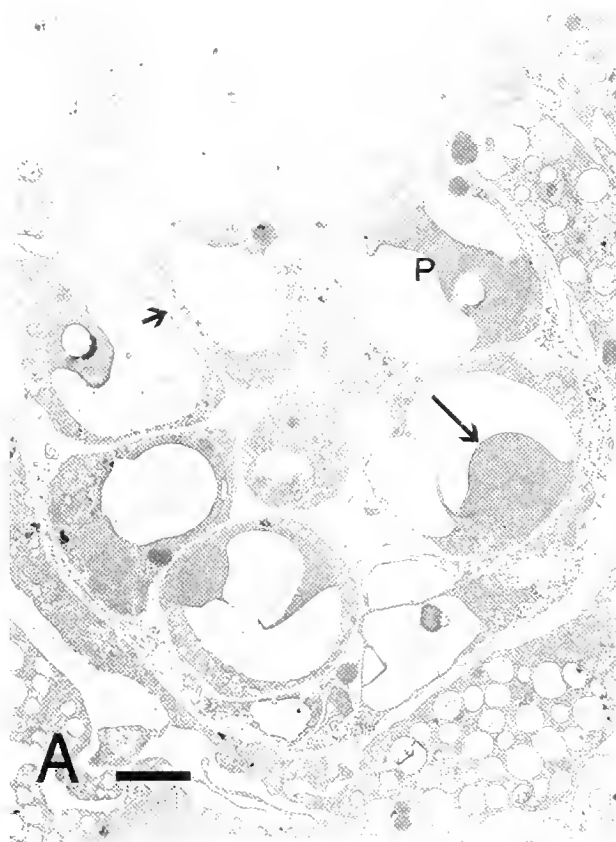


Figure 2. Transmission electron micrographs of *in vitro*-cultured *P. marinus* within hemocytes sampled from *C. virginica* at 2 and 18 h postinjection. Scale bars represent 2 μ m. P = one of six, four, two, or four parasites within a hemocyte in Panels A–D, respectively. Long arrows point to membranes, and short arrows point to cell walls to highlight differences in the integrity of these structures for live-injected and heat-killed injected parasites at 2 and 18 h postinjection. (A) Live injected *P. marinus*, 2 h postinjection. (B) Heat-killed injected *P. marinus*, 2 h postinjection. (C) Live injected *P. marinus*, 18 h postinjection. (D) Heat-killed injected *P. marinus*, 18 h postinjection.

after injection of natural parasites, but the abundance of apparently healthy parasites in the hemocytes of infected oysters as observed by numerous investigators (Mackin 1951, Perkins 1988) and our personal observations suggests that natural parasites might be more resistant to intracellular destruction than cultured parasites. Such a difference may partly explain the apparently low virulence of cultured versus natural *P. marinus*. Clearly, these are preliminary results, but as discussed, they appear to indicate some important differences between cultured and natural parasites while also providing new insight into this host-parasite interaction. We are conducting additional experiments to provide more definitive answers, but caution those using cultured cells to carefully consider the inoculation methods used and the extent to which these cells mimic naturally occurring cells as they interpret their results.

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INDUCED THERMOTOLERANCE IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*

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ABSTRACT Pacific oysters, *Crassostrea gigas*, were subjected to heat shock at various temperatures under controlled laboratory conditions. These experiments demonstrated that exposure to sublethal temperatures dramatically enhances thermotolerance. Oysters exposed to a single nonlethal heat shock (37°C for 1 h) acquired a transient tolerance to a subsequent exposure previously determined to be lethal (43°C for 1 h). The induced thermotolerance ("thermal memory") existed for at least 10 days after sublethal heat shock. Preliminary studies indicated that thermotolerance induction was correlated with the appearance of heat shock proteins in the 70-kD family (hsp-70), based on electrophoretic analysis of proteins from three different tissues, followed by immunoblot analysis with antibodies against hsp-70.

KEY WORDS: oyster, thermotolerance, heat shock protein, summer mortality

INTRODUCTION

Mass mortalities among commercially important oyster species in the United States have become a recurring obstacle for oyster growers since the 1950s. For example, summer mortalities of *Crassostrea gigas* in northern California have approached losses totaling 65% in recent years, (Friedman and Olin unpubl.), and along the East Coast, mortalities of *Crassostrea virginica*, due to documented parasitic infections, have drastically reduced commercial oyster fisheries (Ford 1996, Andrews 1996). Several hydrographic and biological factors have coincided with summer mortality in oyster populations; these include elevated water temperatures, salinity stress, pathogens, and reproductive stress (Perdue 1983, Newell 1985, Beattie et al. 1988, Littlewood and Ford 1990, Friedman et al. 1991, Friedman and Hedrick 1991, Newell et al. 1994).

The ability to adapt to changing environmental conditions is important in all organisms. One of the most studied phenomena is the capacity of different organisms to survive extreme temperatures developed by a short pretreatment at moderately elevated but sublethal temperature. This phenomenon, known as induced thermotolerance, induces resistance against high temperature conditions that would otherwise be lethal (Henle and Dethlefsen 1978, Li and Hahn 1980, Nover 1991, Parsell and Lindquist 1994). Thermotolerance is known to be a widespread phenomenon in organisms and is thought to be an important adaptation to survive changing environmental conditions. Such tolerance-inducing treatments also induce the synthesis of a small number of proteins known as the heat shock proteins (hsp) that play vital roles in allowing the organisms to survive subsequent more severe exposure to heat that would otherwise be lethal (Li and Laszlo 1985, Lindquist 1986). These proteins are involved in the protection, enhanced survival,

and restoration of normal cellular activities in stressed cells and tissues (Subjeck and Shyy 1986, Schlesinger 1990, Hightower 1991, Welch 1991, Gething 1991, Craig et al. 1993, Schlesinger 1994). The synthesis of proteins in the 70-kD family (hsp 70) is correlated with the induction of thermotolerance (Bosch et al. 1988, Nover 1991, Solomon et al. 1991, Weber 1992, Sanders et al. 1994). These proteins are formed not only in response to heat but also are induced in cells and tissues of organisms by a variety of noxious stimuli including anoxia, heavy metal ions, ethanol, and viral agents (Nover 1991). In this study, we determined if the Pacific oyster, *C. gigas*, from two different geographic locations, could acquire thermotolerance. This was accomplished by heat shocking oysters at a predetermined sublethal temperature, followed by exposure to temperatures that were previously determined to be lethal. Evidence is presented that oysters acquired thermotolerance under laboratory conditions, and this induced thermotolerance existed for at least 10 days after the initial heat shock at a sublethal temperature. Preliminary studies indicated that this induced thermotolerance was associated with the expression of hsp-70.

MATERIALS AND METHODS

Collection and Maintenance of Animals

Pacific oysters, *C. gigas*, were obtained from two different seed sources: Kuiper Mariculture, Humboldt Bay, CA, and Dick Poole's Lummi Indian Shellfish Hatchery, Bellingham, WA. Live oysters from both sources were transported overnight on ice to Tomales Bay, CA, in April 1995 (Tomales Bay Oyster Company). Immediately after arrival, oysters were outplanted, on off-bottom

racks in Nytex 1/4-inch mesh bags at the +1.5-ft tide level. After an acclimation period of 4 mo, oysters from both sources were harvested and transported on ice to Bodega Marine Laboratory (BML) where the thermotolerance studies were conducted. On arrival at BML, oysters were transferred to aerated 135-L running seawater aquaria and maintained at ambient temperature ($12 \pm 1^\circ\text{C}$, monitored daily) until used in thermotolerance experiments within 7–8 days of collection. Oysters were measured (shell length), weighed, and placed in fiberglass screen bags (10 oysters per bag). Animals were fed *ad libitum* with a prepared algal diet, Diet C (Coast Seafood Co., Quilcene, WA), diluted to yield a suspension of 100,000 cells per oyster. Feeding was withheld for 24 h before the heat shock and thermotolerance experiments described below.

LT₅₀ Determination

Oysters only from Humboldt Bay were used in the LT_{50} determination. These animals were not acclimated in Tomales Bay. Before any experiments on thermotolerance induction were conducted, we established: (1) the time taken for the core body temperature to reach the target temperature after immersion in a water bath; (2) the range of temperatures over which *C. gigas* survived under laboratory conditions, from which we determined the temperature that resulted in 50% mortality (LT_{50}).

The time for stabilization of the body temperature, after immersion into a water bath set at a desired temperature, was monitored with an Omega thermocoupler probe (Fisher Scientific). A 1-mm-diameter hole was drilled in the shell through which the thermocoupler probe was inserted into the body cavity. The hole was then sealed with Dow Corning high-vacuum grease, after which the animal with the inserted thermocoupler was immersed into a water bath (Masteline Forma Scientific, Model 2095) that contained 4 L of seawater previously heated to 44°C . Oysters were immersed so that the thermocoupler was not in contact with the water. The internal body temperature was recorded every 30 sec, and the time taken for the body temperature to reach the external (seawater) temperature was determined.

During a pilot study to determine the LT_{50} , oysters were exposed to elevated temperatures that ranged from 25 to 50°C in increments of 5°C . In order to minimize the drop in water temperature when oysters were immersed, a two-step heat shock protocol, using two water baths, was followed. The oysters were immersed for 10 sec in a water bath (Precision, Model 181) set at the desired temperature. After this, the animals were transferred to the second water bath (Masteline Forma Scientific, Model 2095) set at the desired temperature. Oysters were maintained at this temperature for 1 h, agitated for the first 10 min of immersion, and subsequently returned to ambient temperature for 7 days. Mortality was then assessed by examining valve closure and/or assessing the presence of decay. Valves that remained open after the shells were pinched together and then released indicated death.

During the pilot heat shock experiments described above, 100% mortality was observed at temperature greater than 40°C (1 h). In order to determine the temperature that induced 50% mortality (LT_{50}), oysters were exposed to a finer temperature range, 40 – 45°C in increments of 0.3°C , for 1 h as described above. From this study, the LT_{50} was determined to be 42.3°C . This formed the basis for subsequent studies on induced thermotolerance, as described below.

Induction of Thermotolerance

On the basis of results obtained from the LT_{50} studies described above, we selected 37°C for 1 h for the sublethal heat shock and 43°C for 1 h for the lethal shock. To examine acquired thermotolerance, oysters (maintained at ambient temperature of $12 \pm 1^\circ\text{C}$) were exposed to a sublethal heat shock as described above and then returned to ambient temperature ($12 \pm 1^\circ\text{C}$) for recovery. The lengths of the recovery period varied: 5, 10, and 20 days postsublethal heat shock. At the end of each recovery period, oysters were heat shocked at 43°C for 1 h and then returned to ambient temperature and monitored daily. Control treatments included: (1) exposure of oysters to lethal temperature without prior exposure to sublethal temperature; (2) sublethal shock alone, and; (3) no heat shock. Mortality was assessed, as described above, 7 days after each treatment. All experiments were repeated three times, each using 10 oysters per treatment.

Electrophoresis and Immunoblotting

In order to determine if a sublethal heat shock was associated with the induction of heat shock proteins, tissue samples were prepared for electrophoresis and immunoblotting as follows. After sublethal shock at 37°C for 1 h, oysters were maintained at ambient temperature ($12 \pm 1^\circ\text{C}$) for 24 h. At least 3 oysters from each trial (total of three trials, each using 10 oysters) were opened, and the tissues (mantle, gills, and adductor muscle) were excised, blotted with Whatman No. 1 filter paper, and weighed. Samples were homogenized in a buffer containing 5 mM MgSO_4 , 5 mM NaH_2PO_4 , 40 mM HEPES, 70 mM potassium gluconate, 150 mM sorbitol (pH 7.55), and were centrifuged at $1000 \times g$ for 10 min. An aliquot of each sample (supernatant) was analyzed to determine total protein concentration with the Micro BCA Assay kit (Pierce, Rockford, IL). The remaining aliquots were combined with equal volumes of $2\times$ sodium dodecyl sulfate-sample buffer (Laemmli 1970) and heated for 5 min at 100°C . Similar amounts of proteins from various tissue samples, predetermined on the basis of the Micro BSA assay described above, were loaded onto 12% polyacrylamide gels and electrophoresed. After electrophoresis, polypeptides were transferred to nitrocellulose membranes as described by Towbin et al. (1979). Blots were incubated with Tris-buffered saline containing 3% bovine serum albumin ("blocking solution," pH 7.4) and probed with mouse anti-hsp-70 (Affinity Bioreagents, MA3-006) for 90 min at room temperature. The blots were then rinsed in blocking solution ($3\times$, 10 min each), incubated with goat anti-mouse immunoglobulin G (Sigma Chemical Co., St. Louis, MO; Product No. A4416) conjugated to horseradish peroxidase for 90 min, washed, and visualized with 4-chloronaphthol.

RESULTS AND DISCUSSION

Oysters, like other marine invertebrates, are ectotherms; their body temperature, is controlled by ambient temperatures that often change rapidly. In our studies, oysters became isothermic with the elevated ambient temperature up to 44°C (from an initial temperature of $12 \pm 1^\circ\text{C}$) within 7–8 min. (Fig. 1). Changes in oyster body temperature in natural settings may be influenced by factors not present in our laboratory setting. It is known that behavioral regulation of body temperature is usually of most importance during short-term fluctuations in ambient temperature, as might occur on a diurnal cycle; however, long-duration temperature changes pro-

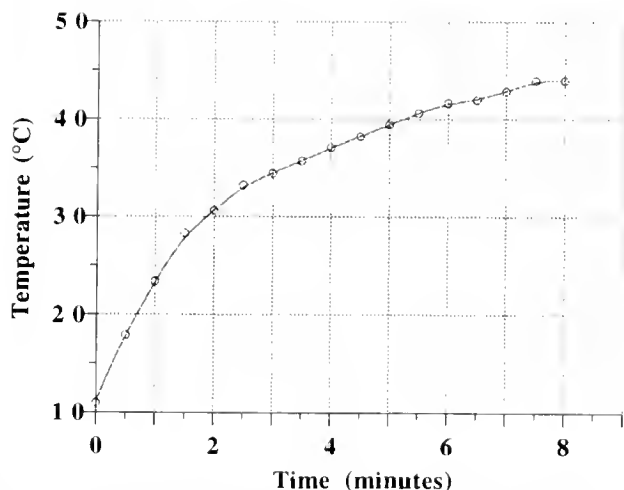


Figure 1. Time taken for the core body temperature to reach the ambient temperature after immersion in a water bath set at 44°C.

vide organisms with enough time for modifications in their biochemical systems (Hochachka and Somero 1984).

Because thermotolerance involves the capability of surviving extreme temperatures developed by a short pretreatment at moderately elevated but sublethal temperature, we first established lethal and sublethal temperatures (Fig. 2). No mortality was observed when oysters were exposed for 1 h to temperatures below 40°C. Direct heat treatments above 43°C, however, always resulted in 100% mortality. The LT_{50} (42.3°C) provided us with the baseline data for further studies on thermotolerance.

Although reared in Tomales Bay for several months, oysters from the two seed sources (Humboldt Bay and Washington State) responded to thermal regimens slightly differently from one another. All oysters from Humboldt Bay that were heat shocked for 1 h at 43°C, without prior sublethal temperature shock, did not survive (Fig. 3). When oysters were exposed to the lethal temperature (43°C) 5 days after a sublethal shock at 37°C, no mortality was observed. Only slightly higher mortality (7%) was observed among oysters exposed to sublethal shock 10 days before lethal shock. The mortality rates of oysters exposed directly to the lethal temperature and of those exposed to lethal temperature 10 days

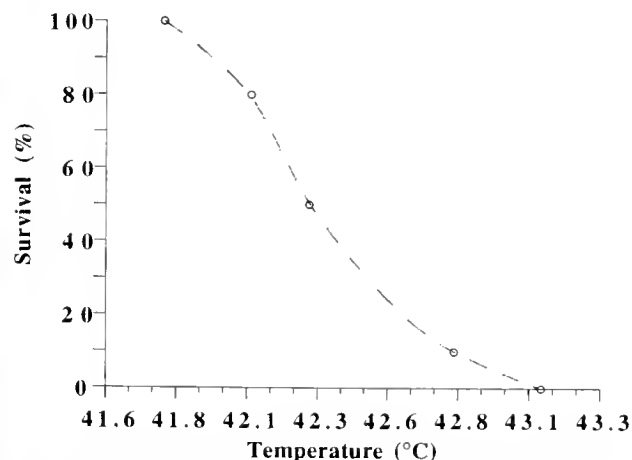


Figure 2. LT_{50} curve for *C. gigas* illustrates the percent survival of oysters 1 wk after exposure to elevated water temperatures.

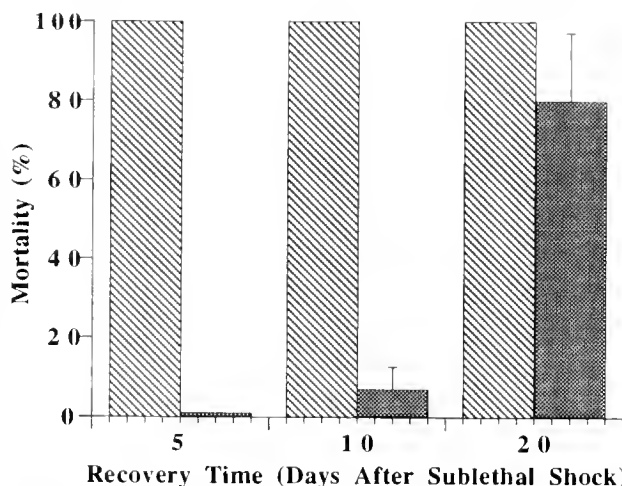


Figure 3. Induced thermotolerance of *C. gigas* from Humboldt Bay. Mortality rates of oysters exposed directly to a lethal temperature of 43°C (▨) or a sublethal temperature of 37°C for 1 h, allowed to recover for 5, 10, and 20 days (at $12 \pm 1^\circ\text{C}$), and shocked at 43°C for 1 h (■). Data represent mean \pm SD for three different experiments, each using 10 oysters per treatment.

after a sublethal shock were significantly different ($p < 0.001$). Twenty days after sublethal shock, however, oysters were not able to withstand the lethal shock and approximately 80% of the oysters died; mortality rate was not significantly different from those exposed directly to lethal temperature ($0.1 < p < 0.25$). In all cases, 100% survival rates were observed among oysters that were either left at ambient temperature ($12 \pm 1^\circ\text{C}$) (data not shown) or shocked at 37°C for 1 h without a subsequent lethal shock. These data indicate that exposure to a sublethal shock followed by a recovery period enhances thermotolerance in these organisms. In addition, the 10-day duration of the enhanced thermotolerance observed in this study exceeds that reported in other organisms under *in vivo* conditions (see Nover 1991 for a review).

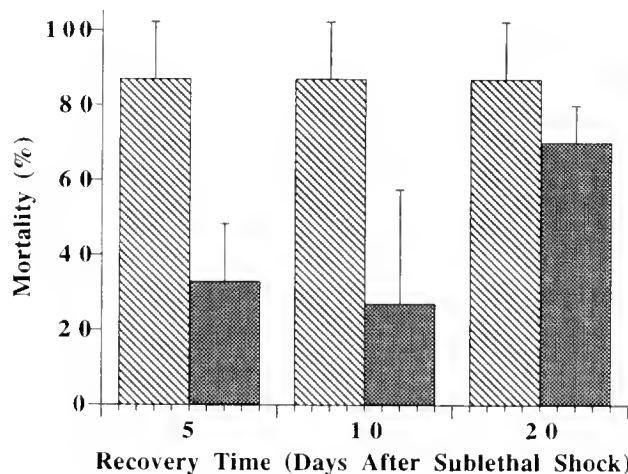


Figure 4. Induced thermotolerance of *C. gigas* from Washington State. Mortality rates of oysters exposed directly to a lethal temperature of 43°C (▨) or a sublethal temperature of 37°C for 1 h, allowed to recover for 5, 10, and 20 days (at $12 \pm 1^\circ\text{C}$), and shocked at 43°C for 1 h (■). Data represent mean \pm SD for three different experiments, each using 10 oysters per treatment.

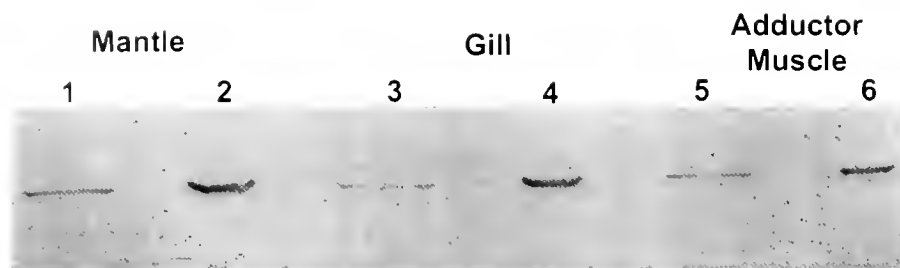


Figure 5. Western immunoblotting to detect proteins of the hsp-70 family in three different tissues of oysters held at $12 \pm 1^\circ\text{C}$ (Lanes 1, 3, and 5) and those exposed to 37°C (Lanes 2, 4, and 6). Similar amounts of proteins from various tissue samples were analyzed on 12% polyacrylamide gels, before probing with anti-hsp-70, as described in the Materials and Methods.

Compared with the Humboldt Bay population, oysters from Washington exhibited less induced thermotolerance (Fig. 4). Complete resistance to a lethal shock was not observed in the Washington State oysters. Approximately 30% of the oysters died after lethal shock 5 and 10 days after sublethal exposure. This was, however, significantly different from the mortality rate observed among oysters directly exposed to the lethal temperature of 43°C ($p < 0.01$). Lethal shock at 20 days after sublethal shock, however, resulted in a mortality rate (70%) that was similar to that of Humboldt Bay (80%). The Washington control oysters that were removed from ambient temperature and immediately given a lethal shock at 43°C , without prior exposure to sublethal temperature, experienced an 87% mortality, which is less than the 100% mortality rate observed in Humboldt Bay oysters given the same treatment. In all cases, 100% survival rates were observed among oysters that were either left at ambient temperature ($12 \pm 1^\circ\text{C}$) or shocked at 37°C (data not shown). The survival rates associated with the induced thermotolerance of oysters from Washington were slightly lower than those observed among the Humboldt Bay population (see Figs. 3 and 4). The reduced mortality in the Washington oysters suggests that different populations and/or strains of oysters may have different thermotolerances. Thus, the LT_{50} should be determined for each group of oysters used in thermotolerance studies. Whether this differential survival rate is due to a geographical or a genetic difference is currently not known.

The data presented above clearly demonstrated the development of induced thermotolerance in Pacific oysters from two different geographical locations in the western United States. Thermotolerance was induced by exposure to 37°C for 1 h, which enabled them to withstand an otherwise lethal temperature (in this case, up to 43°C). The ability of oysters to withstand lethal shock was maintained for a prolonged period of time, for up to 10 days after the initial sublethal shock.

Preliminary studies were conducted to determine if heat shock at a sublethal temperature was associated with elevated expression of heat shock proteins. As shown in Figure 5, heat shocking the oysters at 37°C for 1 h induced the expression of hsp-70; this protein was observed in gills, mantle, and adductor muscle. Although we observed only one isoform of hsp-70, subsequent stud-

ies (unpublished) suggest that there are two constitutive isoforms. Although the immediate role of the heat shock-induced hsp-70 in oysters is currently unknown, previous studies have suggested a correlation between the induction of thermotolerance and heat shock protein expression in a variety of cells and intact organisms (Morimoto et al. 1994, Solomon et al. 1991, Weber 1992, Sanders et al. 1994, Hoffman and Somero 1996, Roberts et al. 1997). The biochemical nature of heat-induced increased resistance to elevated temperature is not completely understood; several lines of evidence have suggested involvement of heat shock proteins (see Lindquist and Craig 1988). Our preliminary data suggest that exposure to sublethal but elevated temperatures enhanced thermotolerance and that an association exists between induced thermotolerance and the expression of hsp-70. As demonstrated in many other organisms (Wynn et al. 1994, Kaufman and Schoel 1994, see also Nover 1991 for a review), the appearance of hsp-70 in oysters (in response to heat shock) may be intimately associated with resistance to other physical and biological stresses in the environment (Lindquist 1986). Whether induced thermotolerance can result in increased survival during summer mortality is currently under investigation. This may be a mechanism adapted by oysters to increase survival under natural conditions. Thus, hsp-70 may be a potential biomarker for stress in oysters. The correlation between the induction of hsp-70 and the decay of thermotolerance in oysters remains to be investigated.

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ALGAL ORGANIC METABOLITES AFFECT SURVIVAL OF PACIFIC OYSTERS, *CRASSOSTREA GIGAS*, LARVAE

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ABSTRACT Cell-free conditioned media obtained from high-density *Heterosigma carterae* isolate Carter cultures caused mortality of larval Pacific oysters *Crassostrea gigas* in 48-h exposures. Streptomycin sulfate added to test vessels prevented mortality at all concentrations of conditioned medium analyzed. These results indicated that bacterial infection of the larvae, and not an algal-produced toxin, was responsible for larval mortality. To explore this hypothesis further, bacteria were isolated from both healthy and diseased larvae. Of the 11 bacterial isolates recovered, 5 belonged to the genera *Pseudomonas* and *Vibrio*, both known to be pathogenic to larval oysters. The *Vibrio* isolate caused mortality when inoculated into healthy cultures of larvae. The growth rate of the bacterium was enhanced by the addition of *H. carterae*-conditioned medium. It was shown that the amount of dissolved organic carbon (DOC) in conditioned medium increased as algal cell density increased and that this conditioned medium significantly influenced the capacity of pathogenic bacteria to divide. Conditioned medium of the cryptophycean alga *Isachrysis galbana* was also shown to produce DOC, although at lower amounts. This observation suggests that many algal sources have the potential to induce both bacterial growth and larval mortality, at differing levels. *H. carterae*-conditioned medium was shown to have no effect on oyster fertilization response, even though it completely inhibited sea urchin fertilization. Further, *H. carterae*-conditioned medium slightly enhanced the fertilization efficiency in starfish, a predator of bivalves.

KEY WORDS: algae, organic metabolites, oyster larvae, antibiotics, bacterial interaction

INTRODUCTION

Algal blooms cause costly seasonal disruptions and even cessation of shellfish harvests worldwide (for reviews see Shumway 1990, Shumway et al. 1990). These blooms can be toxic or noxious, invoking a number of physiological effects on the bivalves (for a review see Shumway 1990). Phytoplankton influence bacterial growth by the release of organics that can act as growth enhancers or inhibitors (for a review see Doucette 1995). The type and abundance of organic matter produced vary over the course of a phytoplankton bloom, leading to a microbial interaction loop (Furhman et al. 1980).

Oyster hatchery operators have long recognized the importance of algal blooms, chlorophyll *a*, and particulate matter as indicators of water quality. Seasonal mortalities of adult and larval oysters have been reported in the northeast Pacific region (Cardwell 1978, Meyers et al. 1990, Friedman et al. 1991), and natural mortalities of adult oysters in south Puget Sound have been correlated with blooms of *Ceratium* (Westly et al. 1966). Hatchery water is routinely filtered, and many hatcheries use ultraviolet light, ozone, chlorine, and antibiotics (Garland et al. 1986, Lewis et al. 1988, Elston 1991, Genthner et al. 1993) to eliminate or reduce bacterial and phytoplankton levels.

In this study, the naturally wall-less raphidophyte, *Heterosigma carterae* (Hulbert) Taylor (ex-*Heterosigma akashiwo*) (Taylor 1992), was tested for toxic effects on oyster fertilization and larval survival. This alga blooms in many areas that also support thriving mariculture industries (Shumway et al. 1990, Honjo 1993). Dramatic changes in marine phytoplankton species composition have been observed during *H. carterae* blooms (Pratt 1966). Experimental studies indicate that *H. carterae* produces allelopathic substances that can have either positive or negative effects on the growth and survival of other organisms. Small aliquots of *H. carterae*-conditioned medium enhanced the growth of the diatom *Skeletonema costatum* (Pratt 1966), whereas large aliquots of the

same medium inhibited the growth of *Skeletonema*. This ectocrine was shown to have short half-life, possibly less than 1 day (Stoney 1990). Wilson (1981) reported that the effects of *H. carterae*-conditioned seawater on the development of echinoderm eggs and plutei ranged from beneficial to lethal. Verity and Stoecker (1982) found only slight effects of *H. carterae*-conditioned media on the growth and survival of the tintinnids *Tintinnopsis tubulosoides* and *Favella* sp.; however, *Favella taraikaensis* and *Synchaeta cecilia* avoided feeding on *H. carterae*, even when the animals were starved (Egloff 1986, Taniguchi and Takeda 1988). Reduced grazing rates were also observed in two species of *Acartia*, juvenile menhaden *Brevortia tyrannus*, and the rotifer *Brachionus plicatilis* (Chotiyaputta and Hirayama 1978, Tomas and Deason 1981, Uye and Takamatsu 1990) when exposed to *H. carterae*-conditioned medium. Not only does *H. carterae* produce an ectocrine that reduced filter feeding in the blue mussel *Mytilus edulis* (Ward and Targett 1989), but the alga has also been shown to be a poor food source for bivalves (Fernandez-Reiriz et al. 1989).

Bacterial-*H. carterae* interaction, and its relationship with other organisms, has been less well studied. Salmonids were killed in xenic *H. carterae* cultures by superoxide production (Yang et al. 1995, Carrasquero-Verde 1997); under the same conditions, axenic cultures were not lethal (Carrasquero-Verde 1997). It is not known if the alga or the bacteria are the superoxide producers.

The nature of compounds produced and released into culture media by *H. carterae* is only partially known. Carbohydrates, such as mannitol, are the major photosynthetic product; glycolic acid and a few amino acids are released in small quantities (Bidwell 1957, Hellebust 1965, Yamaoka et al. 1987, Voronova 1991). A compound similar to the neurotoxin, brevetoxin, has been identified from a Japanese isolate of *H. carterae* (Khan et al. 1997); it is not known if this product is excreted.

The purpose of this study was to determine whether *H. carterae* excretes a compound toxic to oysters. The gametes and larvae of Pacific oyster, *Crassostrea gigas* Thunberg, were chosen as a bio-

assay organism. *C. gigas* is an introduced commercial species of the coastal Pacific waters in which *H. carterae* blooms occur, and the embryonic and larval stages are known to be sensitive indicators of water quality (Woelke 1972). Because an additional potential of an algal toxin is its ability to inhibit fertilization in marine organisms (McGibbon and Moldan 1986), the effect of *H. carterae*-conditioned media on *C. gigas* zygote formation was explored. The relationships between *H. carterae* extracellular products, the natural bacterial flora of *C. gigas* larvae, and the bacterial diseases of the larval were also investigated.

MATERIALS AND METHODS

Algal Culture

H. carterae (Hulbert) Taylor (ex-*H. akashiwo*) (Taylor 1992) isolate Carter was maintained either in O-3, an artificial seawater medium (McIntosh and Cattolico 1978), or in F/2, a supplemented natural seawater medium (Guillard and Ryther 1962). The F/2 medium was modified by reducing EDTA to 0.38 mg/L and omitting silicate. *Isochrysis galbana* Parke was also grown in this modified F/2 medium. All cultures were maintained at 18–20°C. Cells were grown under 70 mEs⁻¹ m⁻² cool white fluorescent light (Philips), on a 12-h light: 12-h dark cycle, with 90 rpm rotary shaking in 2.8-L fernbach flasks that contained 1 L of medium. A ZB-1 Coulter counter with a 100 µm aperture was used to determine algal cell number. Cultures were routinely sampled for bacterial contamination by inoculation of an aliquot into bacterial growth media (Connell and Cattolico 1996), and only axenic cultures were used.

Algal Conditioned Medium

Algae cultured in either O-3 or F/2 medium were sampled over a 28-day growth period. Samples of 100 mL, removed axenically from the culture flask, were centrifuged at 4,000 g for 20 min at 4°C. The retrieved supernatant was then filtered through a GF/C prefilter, followed by passage through a 0.4-µm Nucleopore filter (conditioned 0–3) or through precombusted (4 h at 450°C) GF/C and GF/F filters (conditioned F/2). These filtrates were collected in a sterile receptacle and either used immediately or frozen at –70°C for later use. Heat-treated conditioned medium was prepared by incubating *H. carterae*-conditioned medium at 100°C for 3 min (larval assays) or 10 min (fertilization assay).

Dissolved Organic Carbon

Five-milliliter aliquots of conditioned F/2 medium were used to determine dissolved organic carbon (DOC) levels. Samples from O-3 grown cells were not used in this assay because of the high amount of DOC in the O-3 medium itself. DOC samples were placed in precombusted glass ampoules and stored frozen at –70°C before analysis. Analysis was provided by the University of Washington Ocean Chemistry laboratory.

Larval Culture

C. gigas larvae (110–130 mm) were obtained from the Coast Oyster Co. (Quilcene, WA) or from the University of Washington School of Fisheries, Shellfish Laboratory (Manchester, WA). Oyster larvae were maintained in filtered (0.7 mm pore size) natural seawater (FSW) at 15°C. Seawater was changed and the larvae were fed *I. galbana* (10⁷ cells per 10³ larvae) every 2 days. Nitex

screens (73-mm mesh) were used for concentrating and rinsing the larvae, which were counted with a Sedgwick-Rafter chamber.

Larval Bioassay

A 48-h larval bioassay, similar to that of Woelke (1972), was developed. This assay differed from that of Woelke in that 7- to 9-day-old feeding veligers were used, rather than newly fertilized embryos. Larvae were rinsed with FSW and concentrated by filtration on nitex screens. A final concentration of 20–30 larvae mL⁻¹ was obtained by adding 0.1 mL of the larval suspension to 10 mL of conditioned medium. In general, larvae that were maintained in the laboratory for several days before use showed greater mortality than those that were used immediately on arrival from the hatchery, suggesting that larval health deteriorated under laboratory conditions. To eliminate the possibility of variable larval health, conditioned medium filtrate samples from cultures were frozen after collection and all bioassays were performed simultaneously on a single batch of larvae. All experiments were carried out in 60 × 15 mm tissue culture-grade polystyrene Petri dishes. Samples were incubated at 15°C for 48 h. Viability was estimated by counting 100 larvae with a compound microscope at 125× magnification. The presence or absence of ciliary motion was used to classify the organism as alive or dead. Moribund larvae were scored as alive, although their viability was uncertain. Control vessels contained sterile culture medium, and some experiments included a parallel set of filtrate samples to which streptomycin sulfate (100 µg mL⁻¹ final concentration) was added.

Antibiotic-treated oyster larvae were obtained by incubating rinsed larvae in FSW that contained streptomycin sulfate (100 µg mL⁻¹) for 48 h. The larvae were then filtered, rinsed three times with sterile O-3 medium to remove the streptomycin, and resuspended in sterile O-3 medium. All procedures were accomplished under aseptic conditions. Bioassay vessels were prepared with sterile filtered *H. carterae* O-3-conditioned medium. Controls included untreated larvae in conditioned medium and untreated larvae in conditioned medium to which streptomycin sulfate (100 µg mL⁻¹) had been added. To determine the number of bacteria present in bioassay vessels after the 48-h incubation period, dilutions were plated and the number of resulting colonies were counted.

Fertilization Bioassay

Adult *C. gigas* obtained from the University of Washington Department of Fisheries (Manchester field station) were used to collect gametes by the strip spawning method (Downing and Allen 1987). Eggs and sperm were washed and strained in sterile O-3 media. For comparison, a mussel (*M. edulis*) and three echinoderms (purple sea urchin, *Strongylocentrotus purpuratus*; green sea urchin, *Strongylocentrotus droebachiensis*) as well as a starfish (*Pisaster ochraceus*) were also used for analysis. The experimental animals were obtained from the University of Washington, Zoology Department, or were collected from the coast of Washington State. Gametes were collected according to published methods (Barker 1978, Long and Buchman 1989) and washed in sterile O-3 medium. A Sedgwick-Rafter counting chamber was used to determine egg concentration. *H. carterae* culture concentration was 5 × 10⁵ for all experiments. Gametes were appropriately diluted with sterile O-3 medium and then inoculated into *H. carterae*-conditioned medium, heat-treated *H. carterae*-conditioned medium (boiled 10 min), or control medium (sterile O-3), where they

were maintained for 30 min before the initiation of the fertilization assay. Each sample was then washed three times by centrifugation (2 g in an IEC table-top centrifuge) in 10 mL of sterile O-3 before final resuspension in the original volume (sterile O-3). These bioassays were conducted in six-well polystyrene tissue culture Petri dishes. The fertilization was initiated by mixing the gametes, after which samples were taken at 0 and at 90 min by placing 100 μ L of cell suspension into a 1.5-mL centrifuge tube containing 100 μ L of 2% formalin. Cells were scored under a compound microscope for the presence or absence of the first cell cleavage. Sea urchin embryos were additionally scored for cortical reaction.

Bacterial Isolation and Culture

Bacteria were isolated from infected *C. gigas* larvae that had been maintained in *H. carterae*-F/2-conditioned medium. These larvae were rinsed several times with FSW and then streaked onto agar plates (1.5% Bactoagar made in FSW) that contained 37% brain-heart infusion and 5% yeast extract. Plates were incubated at 24°C for 24 h postinoculation. Liquid cultures were grown in the same medium, minus agar. For a set of experiments, an isolated *Vibrio* species was grown on *H. carterae*-F/2-conditioned medium. Culture tubes containing 5-mL aliquots of appropriately diluted conditioned medium were placed in sterile, cotton-plugged glass culture tubes. These tubes were inoculated with 0.1–0.2 mL of *Vibrio* culture to obtain a final concentration of 10^8 bacteria mL^{-1} . The tubes were incubated with shaking at 25°C for 12 h. Absorbance of the cultures was measured with a Klett-Summerson colorimeter fitted with a blue filter. A Petroff-Hausser counting chamber was used to construct a standard curve for cell numbers versus absorbance.

Bacterial Challenges

Vibrio sp. culture that had been isolated from diseased oyster larvae (see above) were used in these experiments. FSW-rinsed larvae were placed in 10 mL of *H. carterae*-F/2-conditioned medium to which liquid bacterial medium (10^7 mL^{-1} bacteria final concentration) was added. Replicates were conducted with and without 100 $\mu\text{g mL}^{-1}$ streptomycin. Cultures were incubated for 48 h at 18°C before larval survival was determined.

Alternatively, larval survival was monitored in *Vibrio*-conditioned medium. This medium was prepared by centrifuging an aliquot of a liquid *Vibrio* culture for 30 min at 8,000 g. The supernatant was passed through GF/F and 0.4- μm Nucleopore filters. The effect of *Vibrio*-conditioned medium on oyster larval survival was monitored after the shellfish were cultured for 48 h at 18°C. Control vessels contained sterile bacterial growth medium.

Statistical Analysis

Mortality at each concentration of conditioned medium was compared with the corresponding streptomycin point via a *t*-test, assuming equal variances. The results of all other bioassays were compared with an unequal variance *t*-test. Results were pooled when significant differences between trials could be detected. Analysis for fertilization assays were performed with Excel 4.0 (Microsoft Inc.). Results were compared via unpaired *t*-test, assuming equal variance. An α level of 0.05 was chosen as a decisive value for all tests.

RESULTS

Algal Conditioned Medium Compromises and Antibiotic Treatment Enhances Larval Survival

Algal cell density was similar (Fig. 1) in cultures grown in either O-3 or F/2 medium. To determine whether larval mortality is influenced by a compound algae extrude into the medium, larvae were maintained for 48 h in either O-3 or F/2 conditioned media in which *H. carterae* had been grown for 0–28 days (Fig. 2). Oyster larvae exhibited significant mortality when conditioned media from cultures with an algal cell density of greater than 10^5 cells mL^{-1} were used, whereas conditioned media from cultures with less than this concentration had no effect. These data suggest that, as the cell density of the algal culture increases, the cell-free conditioned medium becomes more detrimental to oyster larval survival. Microscopic observation of dead and dying larvae revealed the presence of numerous bacteria.

To provide further information on the relationship between bacterial growth and the effects of conditioned media on larval survival, a series of antibiotic experiments were conducted. When oyster larvae were held for 48 h in conditioned media that contained 100 $\mu\text{g mL}^{-1}$ streptomycin sulfate, mortality was nonsignificant at all algal concentrations of conditioned media tested (Fig. 2). Both F/2 and O-3 grown cultures shown similar results. These data support the hypothesis that filtrate from more dense algal cultures enhances the growth of streptomycin-sensitive bacteria.

Filtrate from dense *H. carterae* cultures (5×10^5 cell mL^{-1}) was heated to 100°C for 3 min to determine whether the compounds released by the alga into the growth medium were heat stable. As seen in Table 1, no differences were observed between heat-treated conditioned filtrate and the unheated control.

To determine conclusively whether streptomycin-sensitive bacteria, normally associated with the larvae, were responsible for larval mortality, bioassays were performed with streptomycin-treated larvae (Table 2). Oyster larvae were maintained for 48 h in sterile FSW that contained 100 $\mu\text{g mL}^{-1}$ streptomycin before the larvae were placed in conditioned medium. Survival of untreated larvae in O-3 medium (controls) was 99%. Survival of untreated larvae in and O-3-conditioned medium was 55%. Streptomycin-treated larvae demonstrated greater than 98% survival in controls and trials. It was evident that larval survival was significantly enhanced when bacterial growth was prevented by the pretreat-

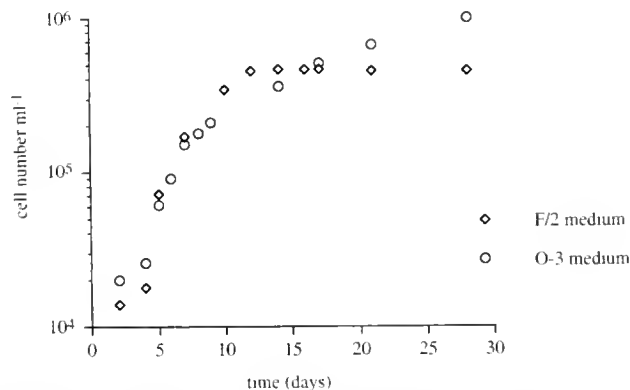


Figure 1. Growth of *H. carterae* in O-3 (circles) and F/2 media (diamonds).

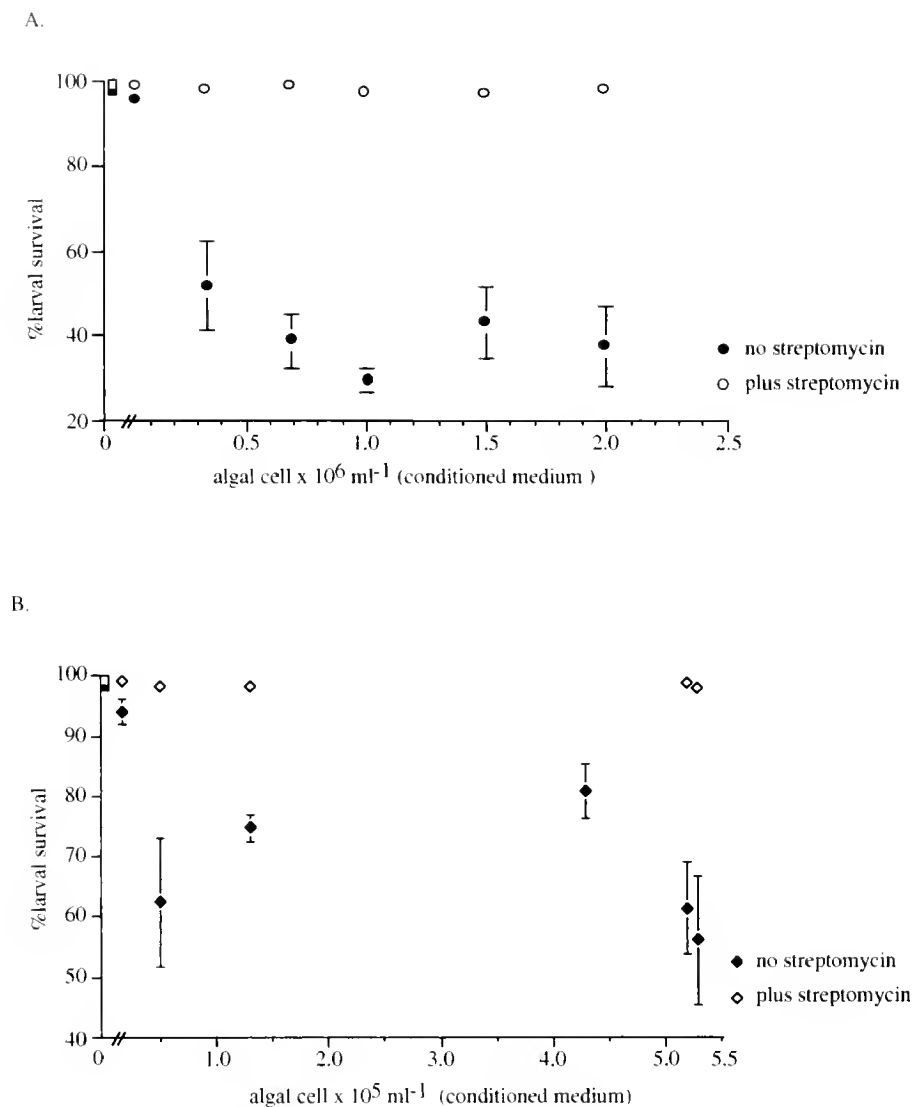


Figure 2. Survival of *C. gigas* larvae after a 48-h incubation in (a) O-3-conditioned (circles) or (b) F/2-conditioned medium (diamonds). O cell mL^{-1} = sterile media, open datum points = conditioned media plus $100 \mu\text{g mL}^{-1}$ streptomycin, closed datum points = conditioned media. Survival in the O-3 controls (squares) were: no streptomycin, 99.3% (standard deviation of ± 0.6), and plus streptomycin, 99.7% (standard deviation of ± 0.6). Survival in the F/2 controls were: no streptomycin, 98.7% (standard deviation of ± 1.2), and plus streptomycin, 99.3% (standard deviation of ± 1.2).

ment in antibiotic. Because all experiments were conducted under sterile conditions, the bacteria present in untreated larval trials must have entered via the larvae themselves. These results further support the contention that *H. carterae*-conditioned medium is not in itself toxic to *C. gigas* and that mortality is induced secondarily.

TABLE 1.

Survival of *C. gigas* larvae in *H. carterae*-conditioned O-3 and heat-treated O-3 medium.

Treatment	% Survival	SD
O-3 control	99.5	0.8
Conditioned O-3	70.8	15.9
Heat-treated conditioned O-3	70.8	10.5

Mean of triplicate vessels. One standard deviation is presented for two trials.

TABLE 2.

Survival of streptomycin-treated and untreated *C. gigas* larvae in *H. carterae*-conditioned O-3 medium.

Treatment	Streptomycin-Treated Larvae		Untreated Larvae	
	% Survival	SD	% Survival	SD
O-3 control	100.0	0	99.7	0.6
O-3-conditioned medium	98.3	1.5	55.7	5.7
O-3-conditioned medium with streptomycin	99.3	0.5	99.0	1.7

H. carterae culture of 2.2×10^6 cells mL^{-1} . Mean of triplicate vessels.

through proliferation of larval-associated bacteria. Differences among experiments in percent survival of untreated samples may reflect initial larval bacterial load.

Resident Bacteria Kill Larvae

Of the 11 bacterial colonies isolated from both live and dead *C. gigas* larvae, 4 were assigned to the genus *Pseudomonas* and 1 was assigned to the genus *Vibrio* (University of Washington Department of Clinical Microbiology). The remaining six isolates were not conclusively identified. To determine whether the *Vibrio* isolate was capable of causing mortality, this bacterial isolate was inoculated into cultures of healthy oyster larvae. The challenges resulted in 21–27% mortality in three separate trials (pooled data in Table 3). Control larvae, which received the bacterial inoculum plus streptomycin sulfate, suffered less than 1% mortality. Larvae were treated with bacterial growth medium conditioned by *Vibrio* to determine whether a bacterial exotoxin might be present. These larvae exhibited considerable mortality in the *Vibrio*-conditioned medium (Table 3). However, the addition of streptomycin reduced mortality to less than 5%.

Organic Compounds Augment Bacterial Growth

To determine the total amount of carbon-containing compounds present in *H. carterae* culture filtrates, it was necessary to grow the algae in F/2 medium. O-3 media has a prohibitively high background level of DOC, which obscures the contribution made by the growing algal cells. Concentrations of DOC in F/2 *H. carterae*-conditioned filtrate increased with culture density (Fig. 3a). As the cells entered stationary growth ($4 \times 10^5 \text{ mL}^{-1}$), DOC continued to increase steadily from 9 to 23.38 mg of C L^{-1} in filtrates recovered from high cell density cultures ($6 \times 10^5 \text{ mL}^{-1}$). This abundance of carbon-containing compounds appears to have been available to support bacterial growth.

Colony counts were performed to determine the total number of bacteria that were present in F/2 medium at the termination of conditioned medium bioassays (Fig. 3b). Initial bacterial levels of $6.6 \times 10^2 \text{ bacteria mL}^{-1}$ increased to 2.7×10^3 (F/2 control) to greater than 10^8 mL^{-1} bacteria (conditioned F/2 of $6 \times 10^5 \text{ mL}^{-1}$ cells) after the 48-h incubation. Parallel test vessels that contained streptomycin sulfate had less than $2 \times 10^3 \text{ bacteria mL}^{-1}$. These results indicate that the growth of bacteria during the bioassay was proportional to the DOC level of the conditioned medium, and as seen above, DOC levels are directly correlated to algal cell density.

H. carterae-Conditioned F/2 Supports Slow Growth of *Vibrio*

To determine whether *H. carterae*-conditioned F/2 serves as a good medium for supporting bacterial growth, filtrate samples

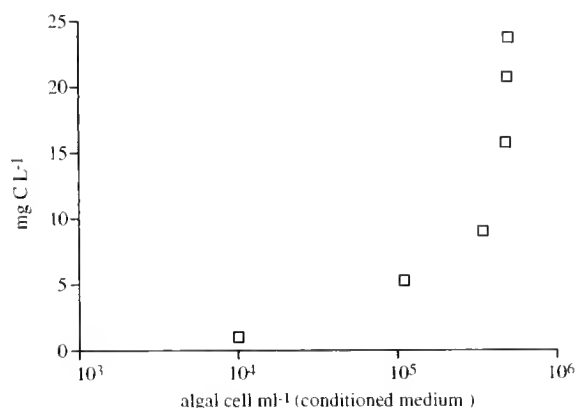
TABLE 3.

Survival of *C. gigas* larvae challenged with *Vibrio* and *Vibrio*-conditioned medium.

Treatment	% Survival
<i>Vibrio</i> inoculum	76.6
<i>Vibrio</i> with streptomycin	99.7
<i>Vibrio</i> -conditioned medium	78.3
<i>Vibrio</i> -conditioned medium with streptomycin	95.7

Mean percent survival for three pooled experiments.

A.



B.

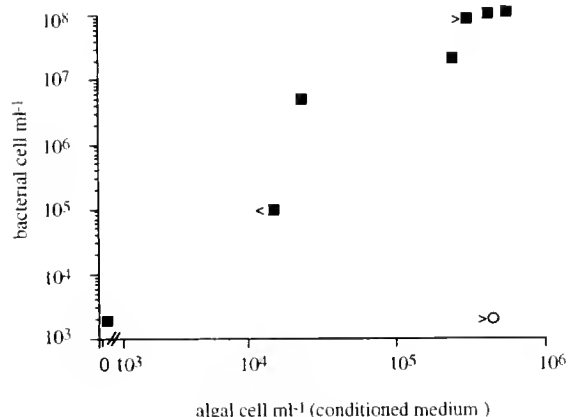


Figure 3. *H. carterae*-conditioned F/2 medium production of DOC and enhancement of bacterial growth. (a) Dissolved organic carbon in *H. carterae*-conditioned F/2 versus algal cell density. (b) Bacterial density in oyster larval bioassays of *H. carterae*-conditioned F/2, as determined by colony count. 0 cells mL^{-1} = F/2 control, open datum points = conditioned medium, closed datum points = conditioned medium with 100 $\mu\text{g mL}^{-1}$ streptomycin sulfate. Greater than and less than (>,<) symbols indicate closest approximations.

were inoculated with *Vibrio* sp. Growth of the bacterium was low at all concentrations of conditioned medium tested (data not shown). Division rates ranged from 0.65 to 1.91 divisions per day. Increasing rates of division were not observed on filtrates from higher-density samples. Very little bacterial growth was observed on sterile F/2 control medium. These data demonstrate that *H. carterae*-conditioned medium alone was a poor substrate for the growth of this *Vibrio* isolate.

Larval Mortality Is Induced by Other Algal Species

To determine whether *H. carterae*-conditioned medium is unique in its ability to induce oyster larval mortality, *C. gigas* larvae were tested with F/2 medium conditioned by the growth of *I. galbana*. Conditioned medium from a high-density ($5.75 \times 10^6 \text{ mL}^{-1}$) culture caused 35% mortality (Table 4). The addition of streptomycin sulfate reduced mortality to 1%. Control larvae in F/2

TABLE 4.

Survival of *C. gigas* larvae in *I. galbana*-conditioned F/2 medium.

Treatment	% Survival	Bacterial cell mL ⁻¹
F/2 media	97.3	5.2×10^2
<i>I. galbana</i> -conditioned F/2	65.0	5.8×10^7
<i>I. galbana</i> -conditioned F/2 with streptomycin	99.0	<10 ³

Bacterial number present in the *Isochrysis*-conditioned medium (with and without streptomycin) at the end of 48-h oyster larval bioassay incubation. The axenic *I. galbana* cultures of 5.75×10^6 cells mL⁻¹ and 4.9 mg L⁻¹ DOC.

medium exhibited 3% mortality. Bacterial density of the bioassay vessels was measured after a 48-h incubation. Although F/2-conditioned test vessels had an average of 5.8×10^7 bacteria mL⁻¹, samples that contained conditioned F/2 medium plus streptomycin had less than 10³ bacteria mL⁻¹ and F/2 controls had only 5.2×10^2 bacteria mL⁻¹. The *I. galbana*-conditioned medium had a DOC value of 4.79 mg of C/L. Thus, this alga also releases a relatively large amount of carbon, which may be used by bacteria, although lower than that produced by *H. carterae*. These data indicate that the ability of *H. carterae* to promote bacterial growth and larval disease is likely shared by many other algal species, even those currently used as food sources for oysters.

Conditioned Medium Differentially Affects Invertebrate Zygote Formation

Sea urchin and mussel fertilization have been used as a bioassay systems for routine water testing of toxicants (McGibbon and Moldan 1986, Long and Buchman 1989, Cherr et al. 1990). The ability of *H. carterae*-conditioned medium to block formation of zygotes was tested with *C. gigas*, *S. purpuratus*, *S. droebachiensis*, *M. edulis*, and *P. ochraceus*. Before the initiation of fertilization, both eggs and sperm were maintained in *H. carterae*-conditioned medium (cell density of 5×10^5 mL⁻¹), heat-treated conditioned medium, or control medium (O-3). As seen in Table 5, the fertilization percentage among experimentals and control did not significantly differ in *C. gigas* or *M. edulis*; however, fertilization was completely inhibited for *S. purpuratus* and *S. droebachiensis* and slightly, although statistically significantly, enhanced in *P. ochraceus*. Fertilization in the sea urchins did not proceed at all, blocking even the cortical reaction.

DISCUSSION

Phytoplankton Contribute Substances That Enhances Bacterial Growth

Bacterial abundance has been correlated with algal growth (Bell 1983, Krstulovic et al. 1991), where algae represent a primary source of organic nutrients. Wolter (1982) demonstrated that bacteria converted an average of 21% of the total phytoplankton exudate of inner Kiel Fjord to biomass. Bacterial use of the exudate was as high as 65%, depending in large part on dominant phytoplankton species. Investigators have hypothesized that blooms dominated by one species of phytoplankton, producing their characteristic complement of substances, will promote the growth of specific bacterial populations (Krstulovic et al. 1991). Products released by the kelps *Laminaria pallida* and *Ecklonia maxima*, composed primarily of mannitol, were initially used by bacteria normally found attached to the kelp fronds (Linley et al.

1981). Heterotrophic use of mucilage proceeded from simple to complex compounds, with the initial bacterial cocci being replaced by rod-shaped genera and eventually by flagellates and ciliates. Thus, not only are heterotrophic bacteria able to use many algal extracellular compounds, they exhibit substrate specific patterns of use.

The Bacteria, *Vibrio* spp., Have Been Associated With Oyster Mortality

Often, periodic summer mortalities of cultured oyster larvae have been linked to infection of the bacterial *Vibrio* species. Vibriosis of larval oysters is a common hatchery disease, causing rapid and extensive mortality (Brown 1987). Inoculations of healthy larvae with a *Vibrio* isolate, infected larvae, broodstock, or effluent wash from infected juvenile oysters caused significant mortality, indicating that this larval-associated bacterium severely decreases oyster larvae survival (Jeffries 1986, Brown 1987, Stiles and Blogoslawski 1993). *Vibrio* sp. can be grown on defined bacterial media, but these cultures do not produce the metabolite that is toxic to oyster larvae unless the growth medium contains hyoaxanthic and glutamic acid, histidine, or sodium thiosulfate (Brown 1984, Brown 1985). Seasonal presence of *Vibrio* spp. occurs in many coastal areas used for mariculture (DePaola et al. 1990) and is correlated with increased water temperature and decreased salinity (Leibovitz 1979, Kaspar and Tamplin 1993). However, larval mortality persisted after the initial peak period of *Vibrio* sp. concentration had passed (Leibovitz 1979). These results indicate that another disease mechanism had taken over, possibly a cascade in the nutrient loop. *Vibrio* infections have been associated with red tides (Romalde et al. 1990), suggesting that environmental conditions that support a bacterial bloom are also conducive for blooms of nuisance alga such as *H. carterae* (Taylor et al. 1994).

H. carterae-Conditioned Medium Enhances Growth of Larval-Associated Bacteria

Results of this study indicate that compounds produced and released by *H. carterae* provide a nutritive source for the growth

TABLE 5.
Percent first cleavage of selected invertebrates in *H. carterae*-conditioned O-3 medium.

Species	Treatment	% First cleavage
<i>C. gigas</i>	O-3 control	99
	conditioned medium	95
	heat-treated medium	94
<i>M. edulis</i>	O-3 control	91
	conditioned medium	88
	heat-treated medium	N.D.
<i>P. ochraceus</i>	O-3 control	87.2
	conditioned medium	93.3
	heat-treated medium	N.D.
<i>S. purpuratus</i>	O-3 control	100
	conditioned medium	<1
	heat-treated medium	<1
<i>S. droebachiensis</i>	O-3 control	94
	conditioned medium	<1
	heat-treated medium	<1

H. carterae-conditioned O-3 medium and heat-treated *H. carterae*-conditioned O-3 medium. *H. carterae* cultures of 5×10^5 cells mL⁻¹. Pooled results of at least four experiments.

of bacteria normally associated with oyster larvae. DOC in the *H. carterae*-conditioned F/2 increased substantially with age and density of the algal culture, providing a large pool of potential nutrient to the bacteria. Growth of larval-associated bacteria (mixed species) during the 48-h bioassay of the *H. carterae*-conditioned F/2 was rapid. The total number of bacteria present after 48 h increased with DOC level of the filtrate sample. These results suggest that this group of bacteria, of partially unknown identity, is able to effectively use the *H. carterae* exudate in the presence of the oyster larvae. It is possible that the larvae themselves, or the other bacteria present, supply an additional nutrient that is used by the assemblage.

H. carterae-Conditioned Medium Is Not Sufficient To Significantly Enhance *Vibrio* Growth

When the *Vibrio* isolate was inoculated into the *H. carterae*-conditioned F/2 medium, low bacterial growth rates resulted, with no increase on filtrates containing higher DOC concentrations. These data indicate the *H. carterae*-conditioned medium was lacking in some essential nutritional compound. The products supplied by unialgal cultures were sufficient for slow bacterial growth but not for bacterial bloom or bacterial toxin production.

Vibrio sp. are not the only bacteria implicated in oyster larval mortality. In two studies designed to determine the usefulness of filtering seawater in aquaculture facilities, larval mortalities resulted from mixed bacterial populations that did not include *Vibrio* spp. (Garland et al. 1986, Lewis et al. 1988). Filtration of the seawater used in microalgal growth medium reduced the number of fatal bacterial infections in the hatchery (Lewis et al. 1988); this measure appeared to have been counterproductive when used in larval growth chambers (Garland et al. 1986). In the second study, larvae failed to develop normally in filtered seawater, even though their mortality rate from bacterial infection was lower than that of the raw seawater group (Garland et al. 1986). These results have led to the hypothesis that the oyster larvae may require metabolites produced by bacteria (Garland et al. 1986).

I. galbana-Conditioned Medium Also Enhances Bacterial Growth

Our data demonstrate that *I. galbana*-conditioned medium affected oyster larval survival in much the same way as *H. carterae*-conditioned medium. Larval mortality resulted secondarily, through an increase in bacterial growth that was supported by the presence of algal-conditioned medium. Undoubtedly, other algae may also be capable of causing larval bacterial disease, potentially through the release of organic compounds.

An Allelopathic Substance Produced by *H. carterae* Effects Invertebrate Fertilization

Allelopathic and toxic substances are often identifiable through fertilization assays (McGibbon and Moldan 1986). Extracts of the

brown algae *Fucus vesiculosus* and *Stypopodium zonale* produce a compound that inhibits first cell division in sea urchins embryos and blocks cytokinesis in mammalian cell cultures (Branham 1963, O'Brien et al. 1989). Additionally, *H. carterae*-conditioned medium has been shown to cause malformation of *Psammechinus miiariis* larvae when applied immediately postfertilization (Wilson 1981) and completely inhibits fertilization in *S. purpuratus* and *S. droebachiensis* (this study). The activity in *F. vesiculosus* extracts, which also blocks the cortical reaction, was found in the ethanol-soluble fraction and was shown to be similar to tannin (Branham 1963). This compound was not freely excreted into the water column and therefore is not in present *F. vesiculosus*-conditioned media. The ectocrines that *H. carterae* excretes into the conditioned media did not inhibit oysters, mussels or starfish fertilization, yet completely inhibited sea urchin egg development. Interestingly, the starfish fertilization was slightly enhanced by *H. carterae*-conditioned medium. Therefore, it is unlikely that *H. carterae* compounds block universal fertilization pathways.

Blooms of *H. carterae* Affect Sea Urchin and Oyster Fisheries by Different Mechanisms

H. carterae ectocrines excreted over a sea urchin bed during spawning would be expected to lead to class failure. In contrast, an algal bloom proximate to an oyster hatchery water source would affect the larval survival indirectly. Algal blooms most likely contribute a large pool of DOC, which can enter the hatchery despite filtration. Under the high-temperature conditions typical of most oyster hatcheries (25–27°C), and in the presence of larval excretory products, even a small population of bacteria could be expected to multiply rapidly, possibly reaching pathogenic proportions. In this way, bacteria that are normally present at low, non-infective levels could become numerous enough to adversely affect larvae. To ensure larval health, steps must be taken to decrease the bacterial load on the larvae themselves during peak algal bloom seasons.

Finally, many of the previous studies searching for allelopathic substances using *H. carterae* were conducted with xenic cultures. The question can then be raised: are reported effects due to a substance produced by *H. carterae* alone, or are bacteria involved?

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EFFICACY OF THE PESTICIDE CARBARYL FOR THALASSINID SHRIMP CONTROL IN WASHINGTON STATE OYSTER (*CRASSOSTREA GIGAS*, THUNBERG, 1793) AQUACULTURE

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ABSTRACT The pesticide carbaryl is applied to intertidal oyster beds in Washington State to control burrowing thalassinid shrimp. We studied efficacy and found a significant dose response relationship for both ghost shrimp, *Neotrypaea californiensis*, and mud shrimp, *Upogebia pugettensis*. A threshold response was observed, suggesting that reducing the commercial application rate below 5.6 kg ha⁻¹ would decrease efficacy and increase variability of resulting kill, particularly for *Upogebia*. Exposure time (time between application at low tide and flood tide) significantly affected the relationship, suggesting that even lower rates (e.g., 2.5 kg ha⁻¹) could be effective when exposure time is sufficiently long (>2–3 h). Typical oyster beds are exposed from 2 to 6 h during minus spring tides. Carbaryl persisted slightly longer (40–45 days) in muddier substrate where *Upogebia* is present than in the well-drained sand inhabited by *Neotrypaea*. However, given rapid initial decline after application (<1 ppm in 24 h), reducing application rate would not greatly influence persistence at levels toxic to nontarget species. This study suggests that growers should be aware of the species of shrimp present on individual oyster beds, because *Neotrypaea* causes much higher initial oyster seed mortality than does *Upogebia*. No oysters survived beyond 300 days on untreated or treated plots where *Neotrypaea* was present. Because of seasonal recruitment of postlarvae to the estuary in late summer and early autumn, *Neotrypaea* is also able to reinfest treated plots immediately, suggesting that long-term control for this species is more problematic.

KEY WORDS: carbaryl, burrowing shrimp, *Neotrypaea californiensis*, *Upogebia pugettensis*, oyster pests, estuary

INTRODUCTION

The ghost shrimp, *Neotrypaea californiensis* (Dana, 1854), and the mud shrimp, *Upogebia pugettensis* (Dana, 1852) (both hereafter referred to by generic name only), dig extensive gallery systems in intertidal and subtidal soft sediments and are widely distributed and abundant in estuaries along the Pacific Coast of North America (Stevens 1928, MacGinitie 1930, 1934, Swinbanks and Murray 1981, Griffis and Suchanek 1991). Burrow construction and feeding activities of these shrimp have important consequences for the structural characteristics of the substrate (Bird 1982, Tudhope and Scoffin 1984) and, consequently, determine the community of organisms that can coinhabit the bioturbated environment (Brenchley 1981, Bird 1982, Posey 1986a, Posey et al. 1991, Brooks 1993, Dumbauld 1994, Simenstad and Fresh 1995). Aquaculture operations and particularly on-bottom oyster culture (*Crassostrea gigas* Thunberg, 1793) require substrate that is sufficiently firm (compact) to support the weight of growing oysters and prevent mortality from sinking and suffocation. Burrowing activity by the shrimp reduces substrate compaction, causing oysters to sink into the altered substrate or to be smothered by sediment that is resuspended during burrow excavation or feeding (Stevens 1928, Loosanoff and Tommers 1948, Washington Department of Fisheries [WDF] 1970, Buchanan et al. 1985, Murphy 1985).

Beginning in the late 1950s, the Washington State oyster in-

dustry experienced problems with reported proliferation of burrowing shrimp populations. Since 1963, the industry has addressed the problem by applying the pesticide carbaryl (1-naphthyl *n*-methyl carbamate, brand name SEVIN) directly to shrimp-dominated intertidal areas when exposed during spring low tides (WDF 1970). Carbaryl is a broad-spectrum organocarbamate that has been widely used for insect control in the terrestrial environment since the use of DDT was banned in 1958, because it does not bioaccumulate in the food chain, has very low mammalian toxicity, and is comparatively short lived (Mount and Oehme 1981, Cranmer 1986). Both carbaryl and its immediate breakdown product, 1-naphthol, inhibit acetylcholinesterase activity, causing nervous system impairment, as evidenced by behavioral changes, paralysis, and death in the shrimp, although the exact mechanism is not well studied (Estes 1986). The practice of applying a pesticide directly to the estuarine tidal flat continues to raise environmental concerns with regard to secondary effects of the chemical on nontarget species and other estuarine resources (Buchanan et al. 1985, Armstrong et al. 1989, Dumbauld 1994). This is the primary reason that the use of carbaryl in estuaries was banned in Oregon (Bakalian 1985), and an environmental impact statement (EIS) was completed on its use in Washington (WDF and Washington Department of Ecology [WDOE] 1985). We completed this study to help resolve several outstanding issues and information needs identified in the EIS: (1) biology of burrowing shrimp and oyster-dominated communities including ecological controlling factors; (2) fate of

carbaryl and its hydrolytic products in estuarine water and sediment; and (3) measures to attenuate nontarget effects, including reduced carbaryl application rate, altered seasonal timing of application, alternative application methods, and pest monitoring and impact assessment. Portions of our results were incorporated in a supplemental EIS (WDF and WDOE 1992).

From the outset of the large-scale shrimp control program in Washington in 1963 until 1984, carbaryl was applied at 11.2 kg ha^{-1} . Tests conducted by WDF in the early 1960s (WDF 1970) showed this rate to be effective and to kill 90–95% of the shrimp on a given bed. With increased use and increased public concern over its use, WDF began experiments to investigate the potential for decreasing the amount of chemical introduced into the environment by reducing the application rate (WDF and WDOE 1992). Another intended goal was to reduce the effect to nontarget species, especially Dungeness crab. These experiments indicated that reduced levels of 8.4 and 5.6 kg ha^{-1} were equally effective, and starting in 1984, the state reduced the permitted application rate to 8.4 kg ha^{-1} . The experiments we report here represent a more detailed exploration of carbaryl application rate, with the explicit objective of linking control to ecology of the two shrimp species (Dumbauld et al. 1996). Although the question investigated was whether carbaryl could be applied at a reduced rate (less than 8.4 kg ha^{-1}) and still effectively control shrimp, the experimental design used allowed this effect to be quantified and modeled for each species of shrimp. This study also provides the first quantified estimate of oyster loss due to shrimp bioturbation.

MATERIALS AND METHODS

All experiments were carried out in Willapa Bay, a large (260-km^2), shallow estuary located along the southwest coast of Washington (Hedgepeth and Obrebski 1981). The majority of the state's oyster production (Conway 1991) and also the greatest use of the pesticide carbaryl to control shrimp occur in this estuary. A series of spray experiments were conducted from July 1988 through summer 1991 with sets of replicate treatment plots located on intertidal flats in either the Palix River subestuary (Stations 1 and 3; Fig. 1) or the Cedar River subestuary (Station 2).

Because experiments were progressively designed to gather more information, the 1989 experiment was most complete, but data from all experiments proved valuable in model development. Each experiment is numbered consecutively and the design is outlined below, but most sampling techniques were consistent between experiments. Because shrimp often exit their burrows after carbaryl is applied (particularly *Upogebia*), areas to be sampled were covered on the day of pesticide application with mesh netting and staked in order to prevent removal of dead or dying shrimp by predators before they could be counted. Exposure time from pesticide application until water covered each plot, air temperature, and sediment temperature were also measured for each plot on the day of carbaryl application. Thalassinid shrimp were quantitatively sampled with a large, stainless steel coring device (40-cm diameter by 60-cm depth) 24 h after spray. Sediment was excavated from the core, sieved (3-mm-pore-size mesh), and sorted for shrimp. The carapace length (CL) of each shrimp was measured from the posterior mid-dorsal margin to the tip of the rostrum and recorded to the nearest 0.1 mm. A smaller core (25-cm diameter by 15-cm depth) and finer sieve (0.5-mm-pore-size mesh) were used to sample newly recruited 0+ shrimp at selected time intervals. Burrow counts were measured with a 40-cm-diameter ring placed

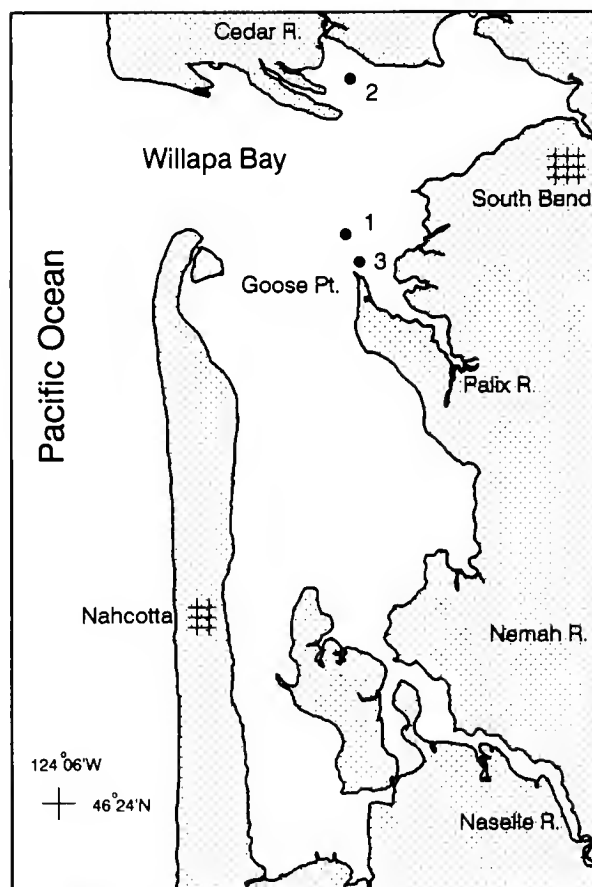


Figure 1. Willapa Bay, WA, showing locations where three carbaryl experiments were carried out. Station 1 was located along the Palix River channel, Station 2 was along the Cedar River channel, and Station 3 was just north of Goose Point.

systematically at four to eight locations within experimental plots as a second measure of prespray and postspray shrimp density. Burrow counts are highly variable by location and season (Dumbauld et al. 1996), and burrow openings of newly recruited 0+ shrimp were generally not large enough to count until the shrimp were ≥ 1 y old.

Experiment 1, 1988

Carbaryl was applied at four concentrations (1.1, 3.4, 5.6, and 8.4 kg ha^{-1}) with a small commercial hand sprayer and tested against a control (no spray). Four sets of replicate plots for each treatment (20 plots total, each 4 m on a side; Fig. 2) were set up at each of two locations in colonies of each shrimp species (near Station 1; Fig. 1). Plots were arrayed in a randomized block design to minimize within-site variation. Shrimp were excavated from each plot with the large core 24–48 h after spray (one sample per plot) and classified as live or dead. Burrow counts were taken on the day of carbaryl application and 1 mo later as another measurement of shrimp density and proportional kill.

Experiments 2 and 3, 1989

A similar experimental design was used in 1989 with four replicate plots for each treatment, but plot size was increased to 10 m on a side (100 m^2) to allow long-term assessment, to reduce

Carbaryl Experiments

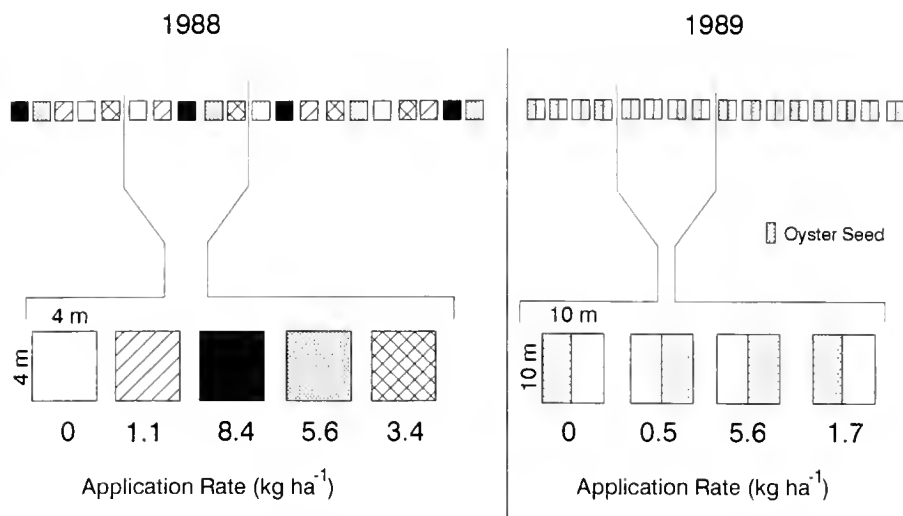


Figure 2. Small-scale carbaryl experiment design. Experiment 1 in 1988 compared the efficacy of four treatments (1.1, 3.4, 5.6, and 8.4 kg ha⁻¹) against a control (no spray). Plots were 4 m on a side. Experiment 2 in 1989 compared three treatments (0.5, 1.7, and 5.6 kg ha⁻¹) against a control, and plots were 10 m on a side. All experiments including Experiment 3 in 1989 and Experiment 4 in 1990 (not shown) were arranged in a similar randomized control block design. Live oyster seed (2.5 bags = 450 shell) was added to half of each plot in 1989 and 1990.

edge effects caused by movement of larger shrimp from adjacent ground, and to permit oysters to be cultured on half of each plot (1989; Fig. 2). Plots were again placed in areas of high shrimp density, with the mud shrimp experiment located along the Cedar River Channel at Station 2 and the ghost shrimp experiment located along the Palix River Channel near Station 1 (Fig. 1). The 1989 experiments were designed to measure shrimp survival at three slightly lower carbaryl concentrations (0.5, 1.7, and 5.6 kg ha⁻¹), based on the results of Experiment 1 (1988). Shrimp were excavated 24 h after application with the 40-cm core to determine initial kill. Samples for newly recruited 0+ shrimp and detailed burrow count estimates were collected on the day of application; at 2 wk; at 1, 3, 7, and 10 mo; and at 1 y after spray. Shrimp samples were excavated with the large core, 1 and 2 y after initial treatment (August 1990 and 1991). Three sediment cores (25-mm diameter by 15-cm depth) were also taken from each of the plots 24 h, 2 wk, and 1 mo after spray. Samples were frozen in the polyvinyl chloride cores, and the top 3 cm was later analyzed by the Washington Food and Dairy Lab for carbaryl concentration by liquid chromatography (Krause 1985; limit of detection, 0.001 ppm).

Because the typical sequence of oyster culture on a sprayed area includes placement of either oyster seed (juvenile oysters known as "spat" on shell known as "cultch") or larger adult oysters on the ground, we planted oyster seed on half of each 100-m² plot sprayed in 1989 (Fig. 2). Seventy bags of oyster seed were purchased from a local oyster company, and 2.5 bags were spread over one-half of each plot in August 1989. We monitored survival of oyster seed and recruitment of 0+ shrimp to both oyster-covered and open halves of each plot. Initial seed density was approximately seven cultch shells m⁻², approximating a commercial planting density. Oyster survival was assessed again 2 mo after planting (10/89), 1 y after planting (8/90), 2 y after planting (8/91), and finally, at a typical harvest size 3 y after planting (9/92). Surviving oysters were counted as clusters (all surviving spat on a shell = one cluster of living oysters) on all plots.

Experiment 3 was carried out between 7/31/89 and 8/2/89 to

verify previous results regarding the magnitude of initial shrimp kill. Sprayed plots were 4 m on a side, like those in Experiment 1, and shrimp samples were taken 24 h after spray with the 40-cm core. Carbaryl concentrations tested (0, 0.5, 1.7, and 5.6 kg ha⁻¹) were the same as those in Experiment 2. No further measurements were made on these plots.

Experiment 4, 1990

One final experiment was carried out in 1990 to confirm oyster survival results in an area with less exposure to physical variables than that chosen for the ghost shrimp experiment in 1989 (where all oyster seed was buried in the first 2 mo). Plots were established in an area of high shrimp density at a more sheltered location in the vicinity of the previous experiments but closer to shore (Station 3 just off Goose Point; Fig. 1). Survival was measured at two carbaryl concentrations (5.6 and 8.4 kg ha⁻¹) and a control (no spray). An additional treatment (2.8 kg ha⁻¹, applied twice in an overlapping pattern) was added to determine whether technique and coverage were important variables for future investigation. Shrimp were excavated 24 h after application with the 40-cm core to determine initial kill, and detailed burrow count measurements were taken on the day of application, 1, 3, and 9 mo and 1 y after spray, along with samples for newly recruited 0+ shrimp, 3 and 9 mo after spray. Shrimp samples were excavated again 1 y after initial treatment in August 1991.

Oyster seed was planted on one-half of each plot in August 1990. Surviving oysters were counted 2 mo after planting (10/90), and shells were excavated 9 mo after planting (5/91), when the majority were found underneath the sediment surface. The depth to which they had been buried was measured in the field, and dead oysters were counted and measured in the laboratory. Finally, a set of glass jars (6-cm diameter by 14.5-cm depth) was buried up to the lip in the sediment within treated (5.6 kg ha⁻¹) and untreated control plots on several occasions and left as sediment traps for a 24-h period. Sediment collected was preserved, sieved through a

500- μm -pore-size mesh to remove large debris, dried, and weighed in the laboratory. Two permanent stakes (marked at 1-cm intervals) were also placed in the center of each plot to measure long-term sediment accretion or erosion.

Data Analysis

All experiments in which carbaryl concentration was a fixed variable were initially set up as randomized block designs in the field to examine the categorical effects of treatment. Although results from each experiment were analyzed separately, it became apparent that a dose response relationship might be established. Several other variables including the total number of shrimp present before treatment (measured as starting burrow count before spray or as the total count of live and dead shrimp 24 h after spray) and exposure time (time from pesticide application until flooding tide covered the area) were also recognized as potentially influential concomitant factors. Some simple dose response models were therefore developed, analyzed with the General Linear Interactive Modeling package for the PC (GLIM 4.0, Aitkin et al. 1990, Crawley 1993), and tested as follows:

$$N_{\text{dead}} = t^{\beta_1} N_{\text{tot}} e^{\alpha + \beta_2 X + d_i \epsilon_{ij}} \text{ or } \ln \left(\frac{N_{\text{dead}}}{N_{\text{tot}}} \right) = \alpha + \beta_1 \ln(t) + \beta_2 X + d_i + \epsilon_{ij} \quad (1)$$

where N_{tot} is the total number of shrimp found (i.e., live + dead) when sampled 24 h after spray, assumed to be representative of the number of shrimp present before pesticide application; N_{dead} is the number of shrimp found dead after pesticide application; X is the dose (both application rate X and carbaryl C_0 measured in the sediment were examined separately when available); t is the exposure time (time after application before the tide flooded); d_i is the block effect (categorical location, $i = 1, 4$); although not shown, interactions were initially tested when this term was in the model; $\beta_{1,2}$ are slope terms; and ϵ_{ij} is the residual error.

After initial attempts to model the data with a normal error distribution, which resulted in significant results but a poor fit to the probability distribution (because of the proportional nature of the data), data were fit directly with the binomial error distribution and a logit transformation or link function ($\text{logit } P = \ln \{P/(1-P)\}$, where P = proportion) in GLIM. Thus

$$N_{\text{dead}} = \text{Bin}(N_{\text{tot}}, P_i) \text{ where } P_i = \frac{e^{\alpha + \beta_1 \ln(t) + \beta_2 X + d_i}}{1 + e^{\alpha + \beta_1 \ln(t) + \beta_2 X + d_i}} \quad (2)$$

Factors were added to the models in a stepwise fashion and retained when they provided the best fit. Standard F tests were performed with the mean deviance values for treatment effects and error. The LC macro provided in the GLIM package, which constructs a profile of the deviance for a specified probability, was also used to calculate both the concentration and the exposure time at which 90% of the shrimp were killed (LC_{90}).

Although burrow count measurements were taken several times after the initial carbaryl application, data were not sufficient to model the effect of time as an explicit variable (i.e., developing terms for natural mortality and recruitment). Instead, data from a single time most representative of initial kill were fit to the above model, substituting burrow counts measured on the day of pesticide application (N_{pre}) for the total shrimp count (N_{tot}) and replacing dead shrimp (N_{dead}) with the number of burrows counted after spray (N_{post}). The binomial error distribution and a logit link func-

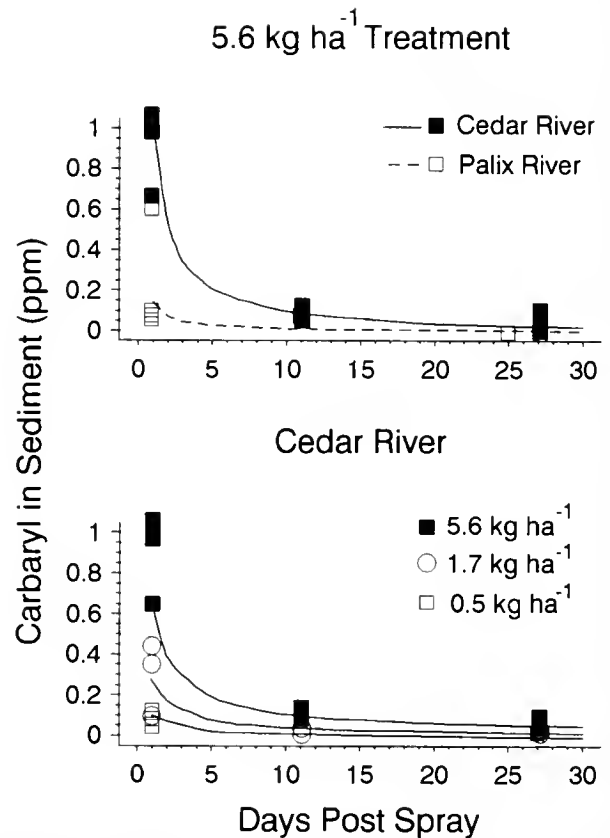


Figure 3. Degradation of carbaryl in the sediment at the Cedar River and Palix River sites (top) and at each of three application rates at the Cedar River location (bottom). Shown are data and lines representing the best-fitting first-order decay rate model (see Table 2). Carbaryl declined more rapidly in the sandy substrate at the Palix River location.

tion were again found to provide a reasonable fit to the model, giving:

$$N_{\text{post}} = \text{Bin}(N_{\text{pre}}, P_i) \text{ where } P_i = \frac{e^{\alpha + \beta_1 \ln(t) + \beta_2 X + d_i}}{1 + e^{\alpha + \beta_1 \ln(t) + \beta_2 X + d_i}} \quad (3)$$

A normal error distribution and log transformation were used to fit a model to data from Experiment 1, where burrow counts actually increased (presumably because of recruitment), causing proportions to be greater than 1 and making it impossible to use the binomial distribution. Minor adjustments were made to a few datum points from control plots in the rest of the experiments, forcing initial proportions to equal 1, and then were fitted with a binomial error distribution.

The concentration of carbaryl remaining in the sediment in Experiment 2 was modeled with a simple first-order decay rate as follows:

$$C_t = C_0^\beta e^{-\alpha t + d_i + s_i \epsilon_{ij}} \text{ or } \ln(C_t) = \beta \ln(C_0) - \alpha t + d_i + s_i + \epsilon_{ij} \quad (4)$$

where C_t is the carbaryl concentration measured at time t (ppm; the lower detection limit 0.001 was added before values were log transformed, when measurements were 0); t is the time (days postspray); C_0 is the concentration measured at time 0 or application rate (X , kg ha^{-1}); α is the decay rate constant; d_i is the block effect (categorical location, $i = 1, 4$); although not shown, inter-

TABLE 1.

Descriptive characteristics of each experiment, location, and general conditions measured on the day of carbaryl application.

Experiment	Date	Station Location	Shrimp Present	Burrows m ⁻²	Approx. Tide Height (m)*	Temperature (°C)			Purpose
						Air	Water	Sediment	
1	7/13/88	1, Palix	Ghost	222	+1.49	na†	14.0–20.0	13.5–19.0	Efficacy/immediate kill
1	7/12/88	1, Palix	Mud	60	+0.03	na	14.0–15.0	14.0	Efficacy/immediate kill
2	7/22/89	1, Palix	Ghost	236	+1.49	19.5	22	20.0	Efficacy/longer term shrimp and oyster survival
2	7/20/89	2, Cedar	Mud	108	+0.46	17.0	19.5	20.0	Efficacy/longer term shrimp and oyster survival
3	8/01/89	1, Palix	Ghost	na	+1.49	na	22.0–24.0	20.0	Efficacy/immediate kill
3	7/31/89	1, Palix	Mud	na	+0.55	na	na	na	Efficacy/immediate kill
4	7/23/90	3, Goose Point	Ghost	398	+1.34	19.0	18.5–21.0	19.0–20.0	Efficacy, coverage/longer term shrimp and oyster survival

* With reference to MLLW = 0.0 m.

† Not measured.

actions were initially tested when this term was in the model; β is the slope; s_i is the station effect ($i = 1, 2$); and ϵ_{ij} is the residual error. Initial application rate (X) was also substituted for C_0 in the model, and interaction terms were tested when appropriate.

The number of oysters left on the surface was modeled with a similar multiplicative function representing survival:

$$N_t = N_0 e^{\alpha t + \beta x + d_i} \epsilon_{ij} \quad \text{or} \quad \ln(N_t) = \ln(N_0) + \alpha t + \beta x + d_i + \epsilon_{ij} \quad (5)$$

where N_t is the number of shells or clusters present at time t ; N_0 is the number of shells placed on the plot at the start of the experiment; t is the time (days postplant); β is the slope; d_i is the block factor (location, $i = 1, 4$); and ϵ_{ij} is the residual error.

Application rate (X) and the number of shrimp burrows (N_{post}) were examined as additional multiplicative independent variables in the model.

RESULTS

General conditions and characteristics of shrimp populations on the day of spray for each experiment are listed in Table 1. All of the *Neotrypaea* sites were located off Goose Point along the Palix River channel at a fairly high tidal elevation ($> +1.2$ m mean lower low water (MLLW)), whereas the *Upogebia* sites were more widespread and were always at lower tidal elevations ($< +0.6$ m MLLW). The substrate consisted predominantly of fine sands (phi size, 2–3) at all locations, but had a higher percentage of very fine sand and silt at the *Upogebia* location ($\bar{x} = 42\%$ at Station 2) compared with the *Neotrypaea* site ($\bar{x} = 10\%$ at Station 3).

Fate of Carbaryl in the Sediments

When applied at 5.6 kg ha^{-1} , carbaryl declined rapidly in the sandy sediment at the Palix River from an average of 0.14 ppm 1 day after treatment to 0.002 ppm 26 days later (Fig. 3), whereas it persisted at slightly higher levels in muddy sediment at the Cedar River (from 1.06 ppm 1 day after treatment to 0.03 ppm , 26 days later). A first-order decay rate model (Equation 4, offset $\beta = 1$) fit the data for carbaryl concentration measured in the sediment reasonably well (overall fit, $r^2 = 0.73$; Table 2) but underestimated the initial rapid decline in carbaryl concentration. A slightly

better fit to the data was obtained when β was estimated with this model. Both station (*Upogebia* vs. *Neotrypaea*) and initial application rate were significant factors in the model ($p < 0.001$, Fig. 3), whereas location within station (block factor) was not. The best fit was obtained when the concentration measured in the sediment 1 day after application (C_0) was used instead of application rate (X) and the block term (d_i) was deleted ($r^2 = 0.87$). Because of the presence of only 3 datum points and lack of data in the initial period after application when decline of carbaryl was rapid, the two endpoints were more influential than might otherwise be the case. Nonetheless, the model indicates that carbaryl could have been present at detectable levels ($>0.001 \text{ ppm}$) for up to 43 days in this experiment, especially in muddy sediments.

Direct Measurement of Shrimp Kill

The proportion of shrimp killed was significantly greater at higher carbaryl application rates, but there were few significant

TABLE 2.

Models and predictive results for carbaryl persistence in the sediments.

Location	Model	Time to 0.001 ppm (days)
Both	$\ln(C_t)^* = \ln(C_0) - 0.15t$	
Station 1	$\ln(C_t) = -4.37 - 0.15t + 0.97 \ln(X)$	24 d
	$\ln(C_t) = -0.98 - 0.14t + 0.81 \ln(C_0)$	28 d
Station 2	$\ln(C_t) = -2.01 - 0.15t + 0.97 \ln(X)$	40 d
	$\ln(C_t) = -0.23 - 0.14t + 0.81 \ln(C_0)$	43 d

Given are a general first-order decay rate model for both stations combined and a first-order decay rate model for each separate location. Models for both dose/application rate (X , kg ha^{-1}) and concentration measured 24 h after spray (C_0 , ppm) as independent variables are given along with the predicted period of time (t , days) it would take for carbaryl to reach an undetectable level of 0.001 ppm .

* C_t , concentration at time t (ppm).

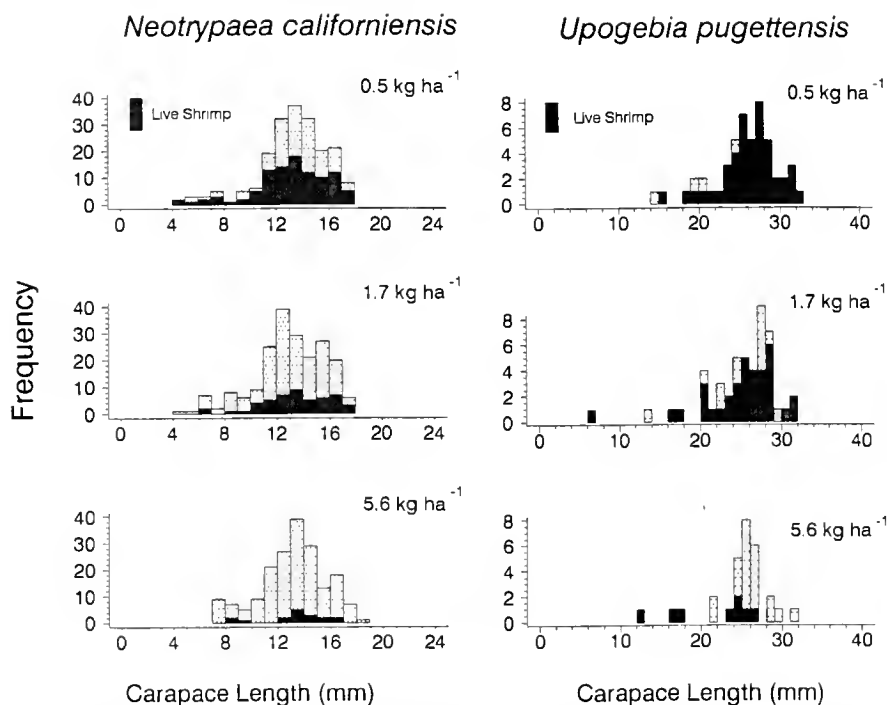


Figure 4. Size distribution of shrimp sampled 24 h after pesticide application in Experiment 2. The number of live shrimp is indicated by solid black bars. Although higher doses increased the kill of both species, there was no apparent difference in the size frequency distribution of dead shrimp as a function of dose.

differences in size distribution of animals killed for either species of shrimp (Fig. 4). In the three cases where statistically significant differences were found (Kolmogorov-Smirnov [KS] test, $p < 0.05$), there were no obvious trends. The total number of animals present (all sizes) and the total number killed were therefore used in all models. Although application rate significantly affected the number of shrimp killed, exposure time was a significant factor as well, especially in the experiments with *Neotrypaea* (Fig. 5, Table 3). Lower concentrations were often as effective as higher doses when exposure time was long ($>2-3$ h). Location within site (block factor) was not significant in any experiment, nor were interaction terms. Several other factors tested, including percent algal, water, and eelgrass cover (not shown here), were insignificant as well. Only exposure time was significant in Experiment 4 when both factors were included in the model and application rate was treated as a categorical factor. Because this experiment was also designed to examine the effects of coverage (comparing 5.6 kg ha^{-1} applied once with 2.8 kg ha^{-1} applied twice), *a priori* tests were run on these data with a similar model and a normal error distribution. Although there was a significant treatment effect, no statistically significant difference could be detected between application rates (2.8 kg ha^{-1} applied twice, 5.6 and 8.4 kg ha^{-1}). It was apparent, however, that the increased coverage provided by effectively doubling the carrier volume (seawater) and spraying the plot twice greatly decreased the variance about the mean proportion killed (from 0.08 at 5.6 kg ha^{-1} to 0.0005 at 2.8 kg ha^{-1} applied twice, Bartlett's test, $p < 0.005$). The minimum application rate necessary to kill 90% of the animals was estimated to range from approximately 2 to 5 kg ha^{-1} for *Neotrypaea* and was higher for *Upogebia* (from 7 to 9 kg ha^{-1}) using the LC macro in GLIM (Table 4). The minimum exposure time ranged from 2 h to just over 5 h for *Neotrypaea* and from 1.5 h to almost 3 h for *Upogebia*.

Burrow Count Measurements

Data taken in the 1989 experiment (Fig. 6) show that the optimal period for assessing effect of the pesticide using burrow counts is about 1 mo after application for both species of shrimp. *Neotrypaea* burrows readily collapsed so that accurate estimates of initial kill could be obtained after 2 wk (one tide series), but *Upogebia* burrows took slightly longer to collapse and disappear. Burrow counts on both control and treated plots for both species began to decline as colder weather approached in the fall, making the effect of carbaryl less discernible 3 mo after spray. By spring of the following year, the effect was still discernible for *Upogebia*, but renewed burrowing activity by a newly recruited cohort of 0+ *Neotrypaea* resulted in similar burrow density on both control and treated plots. Burrow counts taken 1 mo after spray were therefore used in all models to determine efficacy.

Results from dose response models for burrow count were similar to those made for direct kill. Both application rate (Fig. 7) and exposure time contributed significantly to the models (Table 5). Although the error distributions used were different, the resulting models for both *Upogebia* experiments were similar. This was not the case for *Neotrypaea*, where the proportional reduction in burrow openings never exceeded 60% in Experiment 1 and burrows increased on control plots and those treated at low application rates (Fig. 7). Trends for Experiments 2 and 4 were similar but displayed much greater reduction at low application rates. Models were also very similar in the latter case, with both exposure time and application rate contributing significantly to the fit (Table 5). For those models where the LC 90 procedure could be used, results were similar to those from direct kill assessments (Table 4). The minimum application rates necessary to achieve 90% reduction in burrow counts were higher for *Upogebia* than for *Neotrypaea*.

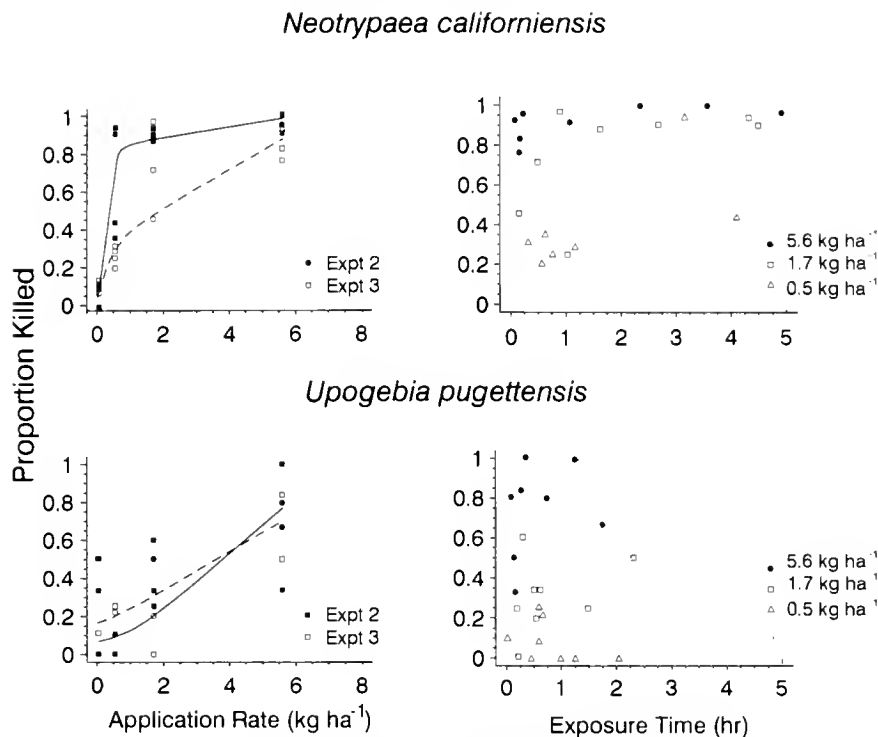


Figure 5. Dose response models for the proportion of *Neotrypaea* and *Upogebia* killed 24 h after spray in Experiments 2 and 3, 1989. Lines represent the best-fitting binomial models (see Table 3). Note the significant rate effect and lower threshold concentration for *Neotrypaea* (left). Exposure time was also significant, with less variability in the proportion killed as exposure time increased, particularly for *Neotrypaea* (right).

Shrimp Reinvasion

One of the primary reasons for expanding the size of the experimental plots to 100 m² in Experiment 2 was to observe the long-term pattern of shrimp reinvasion into areas treated with carbaryl. As noted above, burrow counts taken for 3 y after treatment showed that 0+ *Neotrypaea* rapidly recolonized the treated plots, with burrow density exceeding that on untreated control plots 1 y after spray (Fig. 6). *Upogebia* displayed no such response, and the density of burrow openings on treated plots remained significantly below that on control plots for the 3-y experiment duration. Samples for small postlarval shrimp taken in the plots on 7/31/89, 2 wk after spray, showed that little *Neotrypaea* recruitment had

taken place, although some 0+ shrimp (2–3 mm CL) were present on control plots. Subsequent recruitment occurred in early August, and by the middle of August (1 mo after spray), small shrimp were found in most of the plots. Similar numbers (60–100 0+ shrimp m⁻²) were found in October, indicating that any residual pesticide present did not affect the survival of these shrimp. There was no significant difference in density of 0+ *Neotrypaea* found on treated and control plots (Fig. 8, top), nor was there a significant difference between the density of 0+ shrimp found on the portion of each plot planted with oyster seed (66 ± 20 shrimp m⁻²) and the open side (86 ± 18 shrimp m⁻²). Location (block factor) was marginally significant ($p < 0.10$), with a slightly greater density of recruits found on plots located at one end of the experimental

TABLE 3.

Dose response models for direct kill measurements taken 24 h after spray.

Experiment	Shrimp Present	Treatment	Block	Exposure Time	Final Model*
1	<i>Upogebia</i>	NS, $p > 0.5$	NS, $p > 0.5$	S, $p < 0.001$	$\text{logit } p = -15.68 + 2.08 \ln(t)$
2		S, $p < 0.02$	NS, $p > 0.2$	NS, $p > 0.2$	$\text{logit } p = -1.565 + 0.50 X$
3		S, $p < 0.001$	NS, $p > 0.5$	NS, $p > 0.2$	$\text{logit } p = -2.607 + 0.76 X$
1	<i>Neotrypaea</i>	NS, $p > 0.5$	NS, $p > 0.1$	S, $p < 0.001$	$\text{logit } p = -2.96 + 0.0003 t$
2		S, $p < 0.02$	NS, $p > 0.5$	S, $p < 0.001$	$\text{logit } p = -3.064 + 0.43 \ln(t) + 0.62 X$
3		S, $p < 0.001$	NS, $p > 0.2$	NS, $p > 0.10$	$\text{logit } p = -1.36 + 0.75 X$
4		NS, $p > 0.2$	NS, $p > 0.05$	S, $p < 0.001$	$\text{logit } p = -3.38 + 0.578 \ln(t)$

Given are the results for each term (S, significant; NS, not significant) and the final model chosen in each experiment. All models used a binomial error distribution and logit link function where $\text{logit } p = \ln \{p/(1 - p)\}$.

* X, dose or application rate (kg ha⁻¹); t, exposure time after application (sec).

TABLE 4.

Model results for LC_{90} determination using LC macro in GLIM.

Experiment	Shrimp	Data*	LC_{90}	
			Concentration (kg ha ⁻¹)	Exposure Time
1	<i>Upogebia</i>	Kill	6.7	1 h 18 min
2		Kill	9.0	2 h 48 min
		Burrows	5.0	2 h 30 min
3	<i>Neotrypaea</i>	Kill	7.0	1 h 30 min
1		Kill	—†	4 h 48 min
2		Kill	2.0	3 h 36 min
		Burrows	3.9	5 h 9 min
3		Kill	5.0	2 h 0 min
4		Kill	nt‡	2 h 30 min
		Burrows	nt	2 h 48 min

* Kill data are from direct counts of dead and live animals 24 h after spray, whereas burrows data represent counts taken 1 mo after carbaryl application.

† Model algorithm did not converge.

‡ nt, not testable.

array, and there was also a significant difference in size, with a group of slightly larger shrimp present in the control plots that were apparently present at the time of spray and were therefore killed on the treated plots (KS test, $p < 0.001$; Fig. 8, bottom). Adult shrimp did not appear to reinvade the treated plots in significant numbers, and the 1989 year class of *Neotrypaea* recruits continued to be present and distinguishable, especially on the treated plots, for 3 y after spray (Fig. 9). The effect of application rate was still distinguishable 1 y after spray. Significantly higher densities of older >1+ shrimp were present on untreated plots than those treated at higher rates (0.5 and 0 kg ha⁻¹ > 5.6 and 1.7 kg ha⁻¹; analysis of variance [ANOVA], $p = 0.003$). However, an inverse relationship appeared for 1+ animals (1989 year class), with more shrimp present on plots treated at higher rates (5.6 and 1.7 kg ha⁻¹ > 0.5 and 0 kg ha⁻¹, ANOVA, $p = 0.003$). This appeared to be a density-dependent function, with increased recruitment to plots where shrimp were killed by the pesticide (ln juvenile density = $4.22 - 0.07$ adult density). Neither location (block factor) nor treatment was significant when added to this simple model as long as the density term for adults was present ($p > 0.5$ for both). Recruitment levels of *Upogebia* at the Cedar River location were not nearly as high as those noted for *Neotrypaea* above; however, small cohorts of shrimp <10 mm CL found in control plots were conspicuously absent in the treated plots for all 3 y after carbaryl application (Fig. 10). Adults of this species did not reinvade the treated plots.

Oyster Seed Survival

The survival of oyster seed planted on experimental plots was markedly different between locations. Seed planted in late August 1989 on half of each plot at the Palix site where *Neotrypaea* was present had largely disappeared 2 mo later in October when plots were first revisited. Cultch remaining on top of the sediment surface had declined from a density of approximately 450 shells on the surface of each half-plot (nine shells m⁻²) when planted, to an average of 25 shells present on the surface of plots treated at 5.6 kg ha⁻¹ and only 4 shells on the untreated controls. Seed was found 5–6 cm below the surface of the sediment on many plots. Survival

was much higher at the Cedar River location (*Upogebia*), where counts averaged as high as 387 shells per half-plot in October (8 shells m⁻²; Fig. 11, top). Unexpectedly, seed survived better on control plots than on plots treated with the higher concentrations of carbaryl at this location. By May 1990, there were only a few scattered shells left on the surface of the plots where *Neotrypaea* predominated, whereas counts ranged from two to five shells m⁻² at the *Upogebia* site. Exposure to strong tidal currents along the Palix River channel likely contributed to loss of oyster seed on both treated and control plots in this experiment, yet seed planted at a nearby but more sheltered location inside Goose Point in early September 1990 (Experiment 4) was also lost (Fig. 11, bottom). Treatment was a significant term in models for both species of shrimp (Equation 5), whereas average shrimp burrow density was only important for *Neotrypaea* and block was significant for *Upogebia*. The slope of the relationship with burrow count was negative for *Neotrypaea* and, although not significant, was slightly positive for *Upogebia* (Fig. 12). The slopes of the survival relationships for seed on treated and untreated plots with *Upogebia* present were not significantly different. Because of high initial loss on treated plots, however, more 3-y-old oysters were harvested from untreated control plots ($\bar{x} = 11.6$ clusters m⁻² or 6.5 bushels/plot) than from plots treated at 5.6 kg ha⁻¹ ($\bar{x} = 6.2$ clusters m⁻² or 3.5 bushels/plot) at the conclusion of the experiment in October 1992.

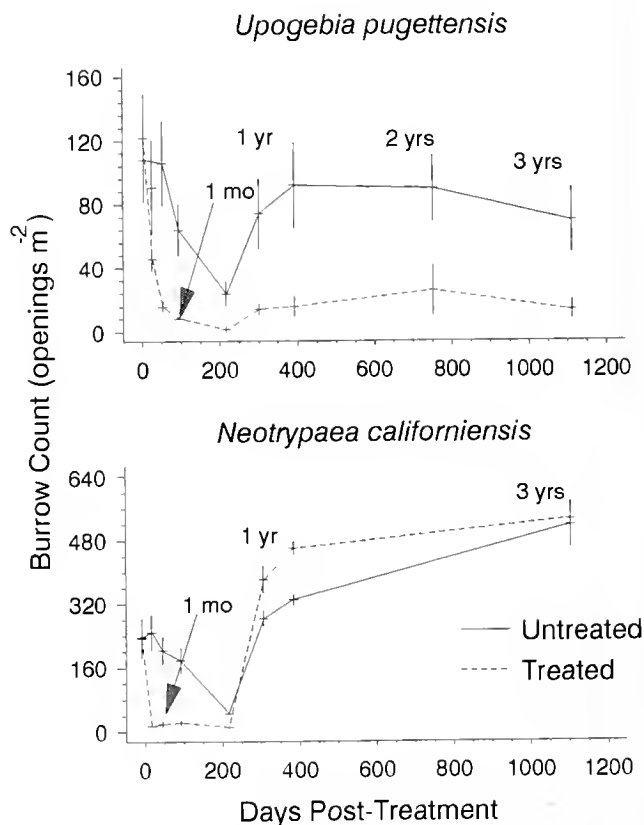


Figure 6. Comparison of average burrow count measurements (mean ± 1 SE) for each species of shrimp taken on untreated control plots and treated (5.6 kg ha⁻¹) plots at selected intervals after carbaryl application in Experiment 2, 1989. Note the rapid decline within the first month on treated plots for both species and rapid recolonization by 0+ *Neotrypaea* (small burrows not counted until 1 y after spray).

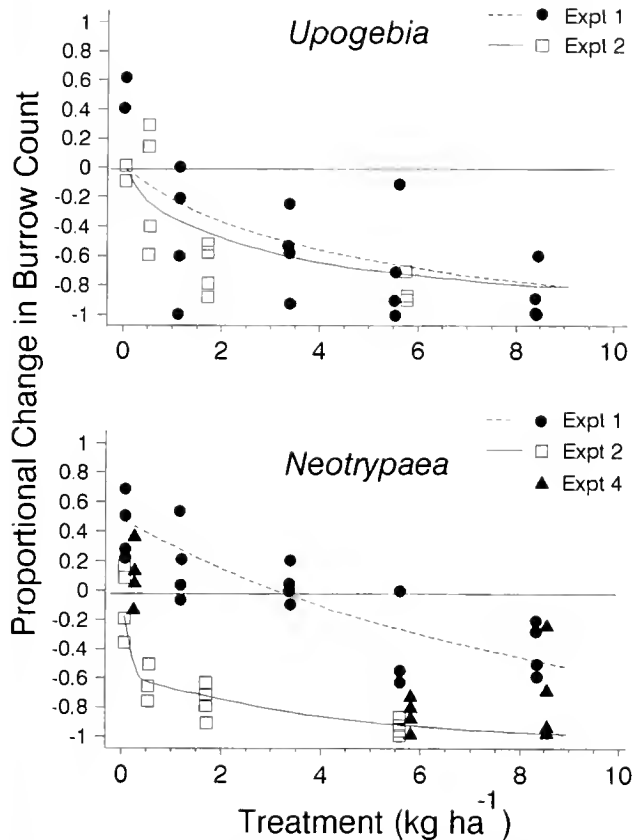


Figure 7. Dose response models representing the proportional change in burrow count for *Upogebia* (top) and *Neotrypaea* (bottom) using measurements taken 1 mo after spray. Trends were similar to those from direct kill measurements (Fig. 5), with a more distinct threshold at lower treatment rates for *Neotrypaea* (except in Experiment 1) than for *Upogebia*. Shown are the best-fitting models (see Table 5) for data from Experiment 1 and 2. Also shown are datum points only (\blacktriangle) from Experiment 4 with *Neotrypaea*.

The amount of sediment collected over 24 h in jars used as sediment traps in control plots ($\bar{x} = 430$ *Neotrypaea* burrows m^{-2}) was significantly higher than that collected in treated plots ($\bar{x} = 43$ burrows m^{-2} , 1990, Station 3), except during October, when it was apparent that high sedimentation rates were prevalent everywhere (ANOVA, $p < 0.001$; Fig. 13). Sediment accumulation rates were, however, markedly different from one day to the next (compare 7/26 with 7/28). There was no statistically significant difference between sediment accumulation in a similar set of jars planted in control and treated plots at the *Upogebia* experiment site (92 burrows m^{-2} on control plots vs. 16 burrows m^{-2} on treated plots, deployed 1 y after spray on 8/26/90 at Station 2). Deposition in untreated *Upogebia* plots was similar to that in the treated plots at Goose Point where *Neotrypaea* had been removed ($\bar{x} = 5$ g of sediment day^{-1}). The depth at which oyster seed was found in the plots at Goose Point the following spring was correlated with both treatment and burrow count (ANOVA, $p < 0.001$). Seed was buried beneath an average of 12.1 cm of sediment on untreated plots, 8.4 cm on plots treated at 5.6 $kg\ ha^{-1}$, and only 6.8 cm on plots treated twice with 2.8 $kg\ ha^{-1}$. A multiplicative model fit the relationship between burial depth and burrow count reasonably well, and block (location) was also a significant factor ($p < 0.001$). Despite the high rates of daily sediment movement and these burial depths for

shell, the absolute sediment level measured between permanent stakes in each plot did not change significantly (1–4 cm accretion). Measurements of dead oyster spat on the shells indicated that those on treated plots had survived and grown from 5 to 10 cm in shell length before being buried.

DISCUSSION

A program based on the use of the pesticide carbaryl to control burrowing thalassinid shrimp on estuarine tidelands for the purpose of growing oysters has been in place for over three decades in Washington (WDF 1970, Buchanan et al. 1985). The effects of carbaryl on nontarget species have been extensively researched (Armstrong and Millemann 1974, Brooks 1993, 1995, Simenstad and Fresh 1995), and a great deal of effort has been expended on regulating its use to prevent such effects on other estuarine biota, particularly Dungeness crab (*Cancer magister* Dana, 1852; Buchanan et al. 1985, Doty et al. 1990, WDF and WDOE 1992). In this study, we attempted to better understand and quantify the efficacy of the pesticide and relate this to the life history and specific effects on the target species of shrimp in the field. Further, despite a wealth of anecdotal evidence and practical experience that dictates that oysters succumb to the shrimp's bioturbating activity (WDF and WDOE 1992), we are aware of no quantitative data and little written documentation specific to oysters before this study.

Efficacy

Results from the experiments conducted in this study suggest that exposure time (time from pesticide application until flooding tide covers an area) is a critical variable that must be considered when evaluating the efficacy of various carbaryl application rates in the field. Neither the initial studies conducted by WDF, which resulted in selection of 11.2 $kg\ ha^{-1}$ (10 lbs $acre^{-1}$) as a commercial application rate (WDF 1970), nor the follow-up experiments conducted in 1985 (WDF and WDOE 1992), which led to reduction in rate for commercial oyster growers, explicitly considered this variable. Several rates (2.0, 5.6, and 8.4 $kg\ ha^{-1}$) were originally tested in 1963, but no further details nor data were given (WDF 1970), making it impossible to evaluate the conclusions drawn. A study by Creekman and Hurlburt reported in the EIS (WDF and WDOE 1992) indicated that seasonal timing of application and temperature were important factors and that a lower application rate (5.6 $kg\ ha^{-1}$) in July and August was as effective as the higher rate of 11.2 $kg\ ha^{-1}$ applied in May and June. They found no significant difference in effectiveness between the rates tested (11.2 , 8.4 , and 5.6 $kg\ ha^{-1}$) in July and August. No exposure times or statistical methods were reported, again making it difficult to evaluate the conclusions drawn. Dose response models using both shrimp mortality estimates made 24 h after spray (Fig. 5) and burrow counts taken 1 mo after spray (Fig. 7) in this study corroborate the lack of significant differences between higher application rates (5.6 and 8.4 $kg\ ha^{-1}$), but also clearly indicate the presence of a "threshold" below which there is increased variability and lack of efficacy (most consistently, <5.6 $kg\ ha^{-1}$ = 5 lbs $acre^{-1}$). Most of the experiments in this study were carried out at typically warm summer temperatures, but temperature may have been a factor causing reduced efficacy against both species in Experiment 1 (Fig. 7, 14–15°C; see Table 1). In general, treatment rate was more important than exposure time for *Upogebia*, and between 7 and 9 $kg\ ha^{-1}$ was necessary to kill 90% of the animals. Exposure time was more influential for *Neotrypaea*, and treatment

TABLE 5.

Dose response models for burrow count measurements taken 1 mo after spray.

Experiment	Shrimp Present	Treatment	Block	Exposure Time	Final Model(s)*
1	<i>Upogebia</i>	S, $p < 0.005$ NS, $p > 0.2$	S, $p < 0.05$ S, $p < 0.10$	NS, $p > 0.2$ S, $p < 0.001$	$\ln N_{post} = 1.19 - 0.26 X + d_i$ $\ln N_{post} = -0.16 - 0.21 \ln(t) + 0.95 \ln(N_{pre}) + d_i$
2		S, $p < 0.001$	NS, $p > 0.5$	S, $p < 0.01$	$\text{logit } p = -4.42 + 0.47 \ln(t) + 0.69 X$
1	<i>Neotrypaea</i>	S, $p < 0.001$ S, $p < 0.005$	NS, $p > 0.5$ NS, $p > 0.5$	NS, $p > 0.1$ S, $p < 0.001$	$\ln N_{post} = 1.72 - 0.16 X + 0.56 \ln(N_{pre})$ $\ln N_{post} = 3.67 - 0.12 X - 0.04 \ln(t)$
2		S, $p < 0.05$	NS, $p > 0.5$	S, $p < 0.001$	$\text{logit } p = -1.64 + 0.20 \ln(t) + 0.50 X$
4		NS, $p > 0.5$	NS, $p < 0.02$	S, $p < 0.001$	$\text{logit } p = -3.56 + 0.642 \ln(t) + d_i$

Given are the results for each term (S, significant; NS, not significant) and the final model(s) chosen in each experiment. Except for Experiment 1, where a normal error distribution was used, all models used a binomial error distribution and logit link function where $\text{logit } p = \ln \{p/(1-p)\}$, and $p = 1 - (N_{post}/N_{pre})$.

* X , dose or application rate (kg ha^{-1}); N_{pre} , number of burrows before application; N_{post} , number of burrows 1 mo after application; t , exposure time (sec); d_i , location (block).

rate could be lower ($2\text{--}5 \text{ kg ha}^{-1}$; Table 5) as long as exposure time exceeded 2–3 h (Fig. 5). This result may be due in part to the lower tidal elevation that *Upogebia* typically inhabits relative to *Neotrypaea*, which is reflected in the sites studied here (Table 1) and generally allows less exposure time for this species. The result may also be linked to the substrate, the shrimp's burrow design, and the behavior of the chemical when it reaches the sediment interface. In the case of the sandy environment where *Neotrypaea* builds an unlined burrow, carbaryl may have a greater chance of directly intercepting the burrow via interstitial circulation than in the muddier environment where *Upogebia* builds a well-lined burrow. Longer exposure times would assure movement of the pesticide in the sand, particularly in areas where water is retained as the tide recedes, but in mud, the pesticide is more likely to be bound to the organic particles and clay at the surface and may not cross the mucus-lined burrow wall. In the latter case, higher treatment rates may simply assure that more pesticide is delivered and reaches the *Upogebia* burrow openings at the surface. Applying the same amount of pesticide, but with more carrier volume in an overlapped pattern (Experiment 4), increased coverage, was highly effective, and reduced the variability in resulting kill.

Chemical Persistence

Persistence of carbaryl in the terrestrial environment is well documented (see Mount and Oehme 1981 and Rajagopal et al. 1984 for review), but information on persistence in the marine environment is less complete (reviewed in WDF and WDOE 1985, WDF and WDOE 1992). Carbaryl is applied by helicopter to oyster beds in Washington estuaries as a wettable powder (particle sizes ranging from 3 to 40 μm) and hydrolyzes slowly in water but more rapidly in the presence of organics, in alkaline conditions, at high temperature, and in the presence of sunlight, forming 1-naphthol, methylamine, several other intermediate breakdown products, and eventually carbon dioxide (Karinen et al. 1967, Lambert and Claeys 1970, Aly and El-Dib 1971, Liu et al. 1981, Larkin and Day 1985). In this study, we compared the rates of breakdown in sandy habitat dominated by *Neotrypaea* and muddier substrate where *Upogebia* is common and related these to any persistent effects on shrimp. Although samples were taken at only three times, a first-order decay rate model suggested that carbaryl persisted in the mud at Cedar River at detectable levels (0.001 ppm)

for up to 43 days after treatment, whereas it only persisted for 28 days in sand at the Palix location (when applied at the rate of 5.6 kg ha^{-1} ; Table 2). Carbaryl levels dropped below 1 ppm in 24 h and 0.2 ppm in the first 5 days (Fig. 3). Karinen et al. (1967) found

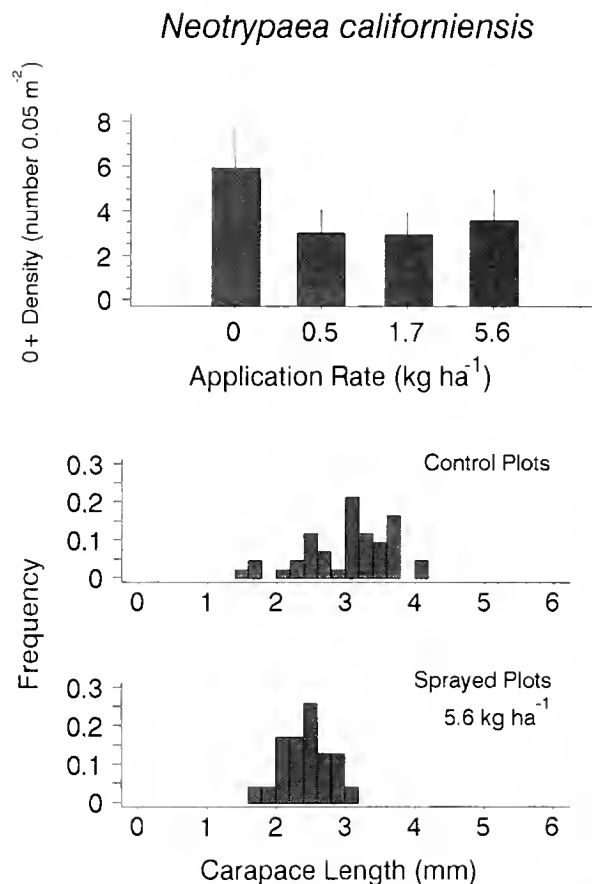


Figure 8. Density of 0+ *Neotrypaea* found on plots at the Palix site 3 mo after spray with different carbaryl application rates (top). A comparison of the length frequency distribution for 0+ *Neotrypaea* sampled on treated (5.6 kg ha^{-1}) and untreated control plots at this time (bottom) indicates 3–4 mm CL shrimp on control plots that were apparently present, but killed by the spray on treated plots.

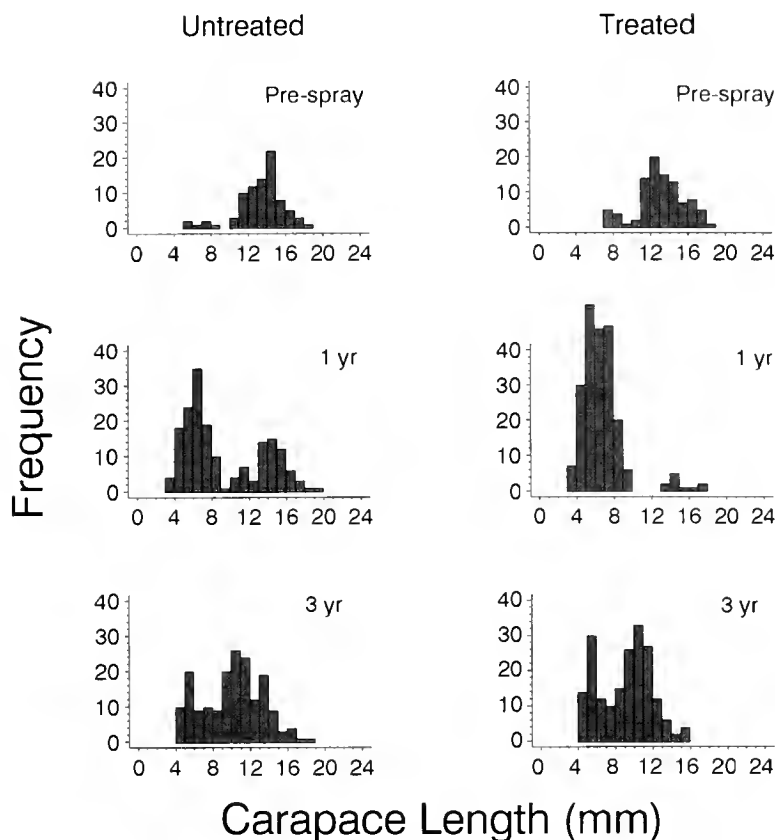
Neotrypaea californiensis

Figure 9. Comparison of length frequency distribution for *Neotrypaea* sampled in treated (5.6 kg ha^{-1}) and untreated control plots at the Palix River before spray, 1 y after spray, and 3 y after spray. Note the lack of movement of older shrimp into the treated plots, but substantial recruitment of juveniles 1 y after spray.

carbaryl present in the mud of Yaquina Bay, OR, at 0.1 ppm up to 42 days after application at 11.2 kg ha^{-1} , but treatments were made during cold weather in February and temperature was shown to be inversely related to hydrolysis in the laboratory studies that they conducted. Data collected by WDF on a larger oyster bed aerially treated with carbaryl at different rates in 1989 (data abstracted from WDF and WDOE 1992) also fit a first-order decay rate model reasonably well [$\ln(C_t) = 1.44 \ln(X) - 0.41 t$; $r^2 = 0.81$]. Together, these results suggest that sediments in the plots sprayed with 5.6 kg ha^{-1} could have remained toxic to shrimp, particularly juveniles (conservative 24-h EC_{50} of 0.01 ppm; see Stewart et al. 1967), for up to 28 days at the Cedar River mud shrimp site and for about 12 days at the Palix River ghost shrimp site. Newly recruited 0+ shrimp were found in the plots within the first month after treatment at the Palix River location, substantiating the fact that the chemical was fairly short lived. A similar effective life for the chemical at the currently used commercial rate of 9 kg ha^{-1} would be slightly longer (15–31 days), assuming that the first-order decay rate relationships above are correct.

Shrimp Reestablishment

Because *Neotrypaea* recruit in the late summer and early autumn (August to October; Dumbauld et al. 1996), reestablishment

of this species can take place almost immediately, even when a high percentage of the adults are removed by pesticide application in July. *Upogebia* recruits in the spring and early summer, however, and did not establish populations on treated plots in subsequent years. Preliminary settlement experiments showed that recruitment of *Upogebia* was influenced by the presence of adults (Dumbauld 1994), and we suspect that removal of adults on the treated plots negatively influenced settlement. Although there are numerous anecdotal reports by oyster growers and bait shrimp fishermen and some documentation that adult shrimp move horizontally in the sediment and in the water column (Posey 1986b, Feldman et al. 1997), no evidence for this behavior was found with respect to the treated areas in our study. The small experimental plots treated here were surrounded by high densities of adult shrimp, yet adults of neither species appeared to reinvade the treated areas to any significant degree (Figs. 9 and 10). Peterson (1984) also found limited reinvansion of plots where shrimp had been removed in California. Larger animals may leave the burrows at certain times of the year (e.g., mating behavior is virtually unknown and they are common prey items of some estuarine fish; Posey 1986b and Armstrong et al. 1995); however, some of the above anecdotal reports are likely the result of observations of greatly increased burrow counts from new *Neotrypaea* recruits invading the open space. We gathered evidence in 1992 and 1993

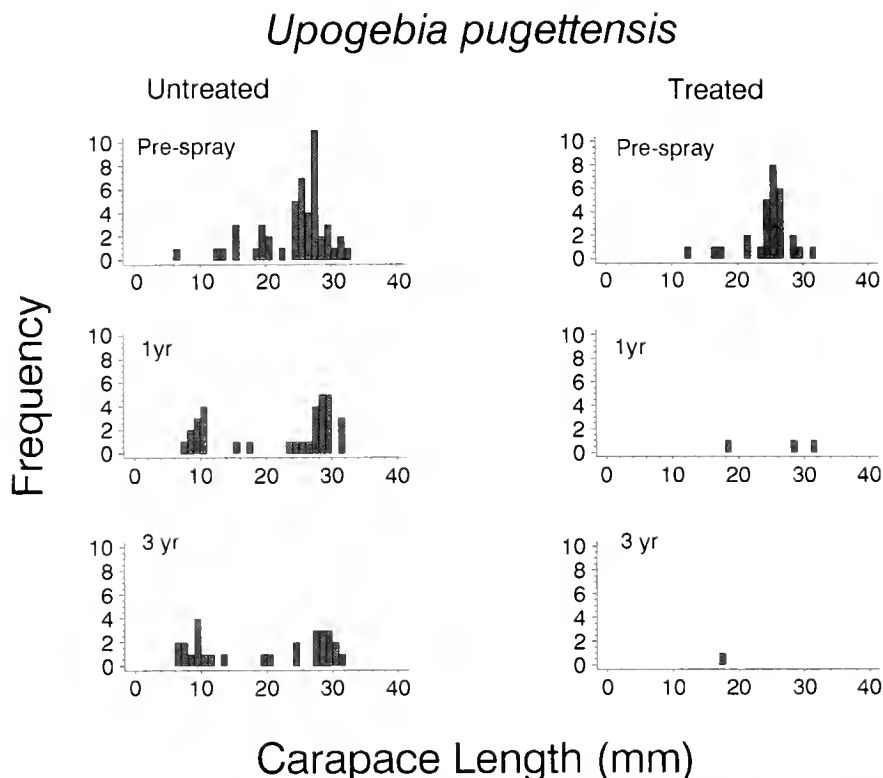


Figure 10. Comparison of length frequency distribution for *Upogebia* sampled in treated (5.6 kg ha^{-1}) and untreated control plots at the Cedar River before spray, 1 y after spray, and 3 y after spray. Note the lack of substantial movement of older shrimp into the treated plots and apparent reduction in recruitment as well.

that a similar pattern of reinvasion by postlarvae occurs on large, commercially treated beds at two locations (Table 6). Burrow counts taken 1 mo after spray indicated that significant reductions in adult shrimp took place on both beds, whereas those taken 1 y after spray showed high levels of recruitment to the bed where *Neotrypaea* was predominant and only limited recruitment to a *Upogebia*-dominated site.

Shrimp Bioturbation and Oyster Loss

Like other species of thalassinid shrimp, both *U. pugettensis* and *N. californiensis* are known to resuspend sediment in the process of burrow construction, maintenance, and feeding (MacGinitie 1930, MacGinitie 1934, Brenchley 1978, Miller 1984, Swinbanks and Luternauer 1987). Quantities reported, however, differ by method of collection and by units used, making comparisons difficult (see Rowden and Jones 1993 for review). Swinbanks and Luternauer (1987) provided the only replicated study and reported $24 \text{ g dry wt shrimp}^{-1} \text{ d}^{-1}$ for *Neotrypaea* but could not measure rates for *Upogebia* using the leveling method in which sediment is collected directly from the surface. Brenchley (1978) reported higher rates for *Upogebia* than *Neotrypaea* in laboratory experiments, but she noted that *Neotrypaea* had difficulty burrowing in the mud that was provided and that both species of shrimp were constructing initial burrows when measurements were taken. Measurements of deposition in this study were taken for comparison only, and not to determine resuspension by individuals, but they confirm previously observed variability of field measurements and suggest that *Neotrypaea* produces significantly higher amounts of

suspended sediment than *Upogebia* on a daily basis (Fig. 13). Although the jars that we used may have significantly underestimated transport because of their low aspect ratio (2.4 to 1, see Emerson 1991), application of the pesticide carbaryl and therefore removal of shrimp caused a significant reduction in the amount of suspended sediment collected.

Increased turbidity due to thalassinid shrimp bioturbation has a direct influence on the benthic community that coexists with shrimp in Willapa Bay (Dumbauld 1994, Brooks 1995) and elsewhere (see Posey 1990 for review). High concentrations of suspended solids are also known to have detrimental effects on growth and survival of lamellibranch bivalves, although some benefit can be derived from lower levels because of the presence of benthic microflora and enhanced filtration rate and preingestive selection (Kiorboe and Mohlenberg 1981, Bricelj et al. 1984, Grizzle and Morin 1989, Grant and Thorpe 1991, Newell and Langdon 1996). Murphy (1985) found that *Neotrypaea* populations in a California embayment resuspended sediment, which had negative effects on survival and growth of the introduced hardshell clam *Mercenaria mercenaria* (Linnaeus, 1758) and also influenced the abundance of other suspension-feeding bivalves. Laboratory experiments indicated that juvenile clams were not only covered by sediment, directly affecting survival, but growth was also inhibited at $23\text{--}29 \text{ mg L}^{-1}$ of suspended particulate matter. Although less detailed work has been carried out on the effect of suspended sediments on oysters, they regulate ingestion in the presence of suspended inorganic matter by producing pseudofeces and are better adapted than some siphonate bivalves to maintain optimal clearance rate in the face of high sediment loads (Jordan 1987,

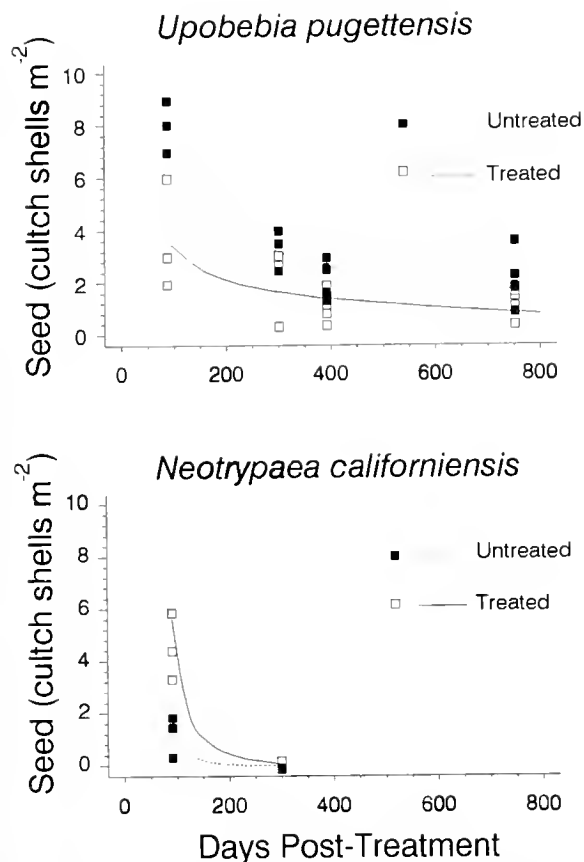


Figure 11. Survival of oyster seed (cultch with attached spat) planted on treated (5.6 kg ha^{-1}) versus untreated control plots in Experiment 2, 1989, where *Upogebia* was present (top), and Experiment 4, 1990, where *Neotrypaea* was present (bottom). There was a significant treatment effect, but shell disappeared below the sediment surface rapidly on both treated and untreated plots when *Neotrypaea* was present. This effect was negative and seed losses were higher on treated than untreated plots when *Upogebia* was present.

Newell and Langdon 1996, Hawkins et al. 1996). Oysters also cease filtering at very high silt levels (Loosanoff and Tommers 1948, Barille et al. 1993).

Because cultured oysters are immobile as small spat on a piece of cultch shell, they are highly susceptible to siltation and burial, whereas at least some clams are not (Emerson 1990). Despite numerous visual reports of oyster losses dating back to diked oyster culture practices in southern Puget Sound (Stevens 1929) and a rather detailed economic assessment of oyster losses claimed by growers (Conway 1991), this study documents reduced oyster survival in the presence of burrowing shrimp and the effectiveness of the pesticide carbaryl in reducing that loss. The small-scale experiments conducted here were carried out under worst-case conditions (small plots surrounded by very dense shrimp beds), making it difficult to apply results to actual oyster culture conditions. However, the initial survival of seed is shown to be the primary determinant of oyster yield and there is a significant difference in the effect caused by each species of shrimp. Oyster seed rapidly disappeared beneath the surface of the sediment, and no oysters survived beyond 9 mo in either of the experiments where *Neotrypaea* were present, whereas survival was much better in the *Upogebia* experiment (Fig. 11). Oysters survived slightly longer on

treated plots than untreated plots at Goose Point, where the amount of sediment deposited on top of the seed was correlated with the density of *Neotrypaea*. A threshold level of approximately 40 burrows m^{-2} , above which seed did not even survive beyond 90 days, was apparent (Fig. 12). Oysters survived better on untreated plots than treated plots at the Cedar River site where *Upogebia* was present. It is suspected that treatment and removal of shrimp and other fauna may have released fine sediments that were bound in the linings of burrows and dense tube mats from coexisting species, causing the initial loss of seed at this location. Results from a recent set of experiments (Feldman and Dumbauld unpubl.), where seed was not planted until the following spring, indicate no difference in initial loss between treated and untreated plots but slightly better long-term survival on treated plots. Increased yield from treated plots at harvest and large seed losses on untreated plots during the first month after planting were also reported in an experiment conducted by WDF in 1986, in which seed was planted on 0.02-ha plots treated at several application rates the previous

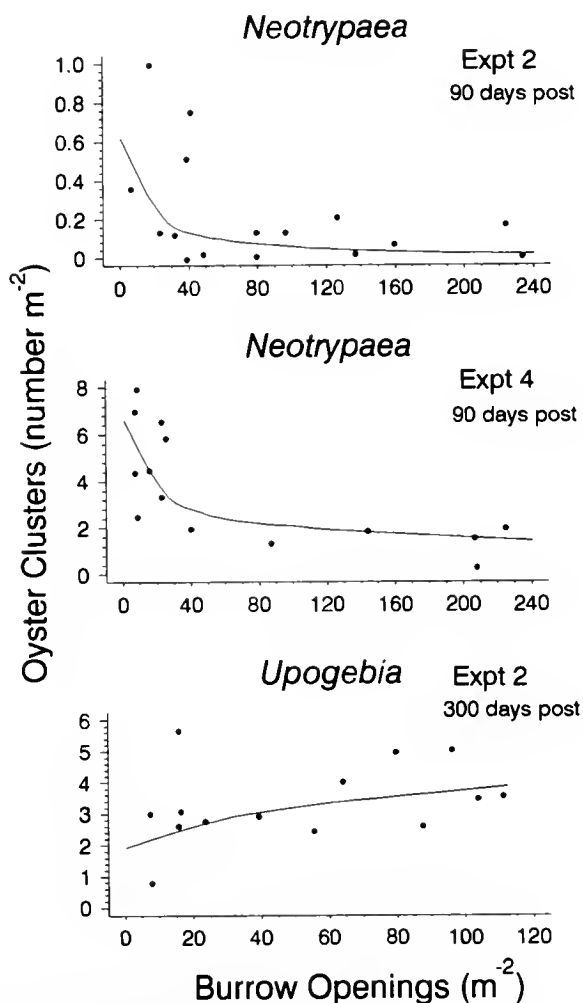


Figure 12. Relationship between shrimp burrow openings and the number of oyster seed clusters remaining on the sediment surface 90 days after treatment. Note the similarity for both experiments with *Neotrypaea* and almost total loss when shrimp burrows exceeded about 40 m^{-2} , whereas a slightly positive but nonsignificant relationship existed between *Upogebia* density and surviving seed.

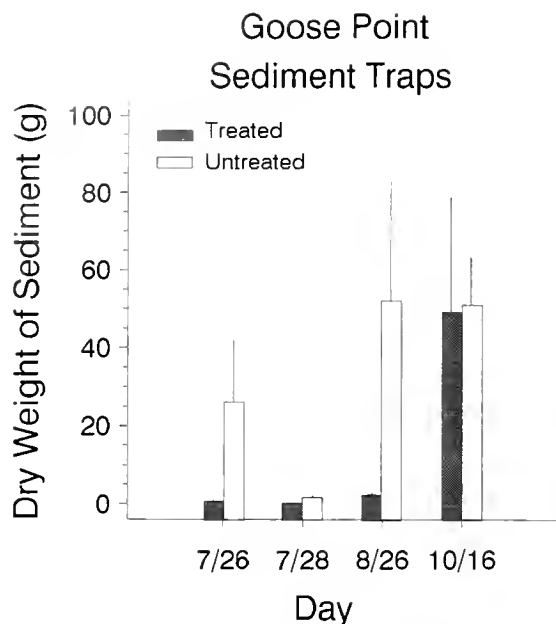


Figure 13. Comparison of the average weight of sediment collected in small sediment traps placed on treated and untreated plots at Goose Point (Experiment 4) 1 and 2 days, 1 mo, and approximately 3 mo after pesticide application. Bars represent ± 1 SE. Although the sediment deposition rate fluctuated dramatically, a significant treatment effect was present in all cases except during October.

year, but no statistical analyses were given (Tufts 1989, WDF and WDOE 1992).

Management Implications and Recommendations

A recent attempt has been made to develop an integrated pest management (IPM) plan for the control of burrowing shrimp in Washington (Burrowing Shrimp Committee 1992) after this became the preferred option selected in a supplemental EIS (WDF and WDOE 1992). Unfortunately, no effective alternative control measures have been discovered to date and the carbaryl-based control effort has remained relatively unchanged since its inception in 1963. An extensive monitoring effort was implemented in the mid-1980s to observe and regulate the potential effect of the carbaryl spray program on Dungeness crab populations and other nontarget organisms, but few attempts have been made to examine the efficacy of the pesticide on the shrimp themselves or the protection afforded the oysters. The small experimental plots and spatial scale of our study were designed to test pesticide efficacy, but we are also able to provide some important initial results and direction for future work on oysters.

Perhaps the most important conclusion to be drawn from this study is that growers should be aware of the species of shrimp present, particularly when beds are to be planted with oyster seed before spray. *Neotrypaea* poses the most significant threat to oyster culture operations and can cause much higher siltation and initial mortality than *Upogebia*. The current density criteria for allowing pesticide application (10 burrows m^{-2}) also seems particularly low for *Upogebia*, and further work should be done to determine when pesticide application is necessary in the presence of this shrimp. Because of seasonal recruitment in late summer and early fall, *Neotrypaea* can also rapidly recruit into beds that have been sprayed, especially given the current scheduling of application in July and August (Dumbauld et al. 1996). There is evidence

that the presence of shell deters recruitment of this species by influencing settlement behavior and increasing the abundance of predators, causing higher postsettlement mortality (Feldman et al. 1997). It seems advisable to ensure that beds have this epibenthic cover present by planting seed or perhaps covering the beds with oyster shell as soon as possible after pesticide application.

Results of this study suggest that reducing the concentration of pesticide applied, which has been suggested as a way of minimizing nontarget effects, is likely to make control efforts less effective, particularly if this rate is reduced below 5.6 kg ha^{-1} and exposure time is short. Reduction in effectiveness is likely to be more significant for *Upogebia*, which appears to be less susceptible to the pesticide, in part because of the lower tidal elevation that it inhabits and correspondingly decreased exposure time to the pesticide. Tidal elevation should always be considered and beds should be sprayed as soon as the tide recedes, assuring maximal exposure time. With the possible exception of decreased offsite effects to nontarget organisms because of more restricted movement of the pesticide, reduction in application rate will not improve the situation for juvenile Dungeness crab, which exhibit virtually 100% mortality when directly exposed to the pesticide in the intertidal at all concentrations (Doty et al. 1990). Experiments indicated that older crab that move up on the intertidal at flood tide are also killed when consuming shrimp poisoned at lower concentrations. In addition, this study suggests that the persistence of carbaryl in the sediments, although slightly longer in a muddy environment (40–45 days), is probably not greatly affected by lowering the application rate, because of extremely rapid initial degradation and loss. Finally, the results of this study indicate that increased efficacy may be achieved by making sure the chemical is delivered accurately and coverage is complete (e.g., increasing the carrier volume and/or the number of passes over a bed). Further experimentation with increased carrier volume is currently underway (Dumbauld pers. comm.).

A final recommendation is for more active monitoring of burrowing shrimp populations themselves and their interaction(s) with commercial oyster operations. If IPM is to be successful, data on the pest and the efficiency of the pest control program are essential. No such data are currently collected in a manner that can be effectively used to improve the program or regulatory decisions that directly influence it. Even though the marine system makes the problem less tractable, current arguments between the oyster growers promoting control and the antipesticide faction involved in this

TABLE 6.
Measurements of shrimp density and eelgrass cover on two commercial oyster beds in Willapa Bay where carbaryl was applied to control burrowing shrimp.

Bed Location	Time	Shrimp Present	n	Burrow Holes (# m^{-2})	Eelgrass Cover (%)
Nemah	Prespray	<i>Upogebia</i>	77	60 (4)	19 (4)
	1 mo post		84	6 (1)	24 (4)
	1 y post		84	14 (2)	32 (4)
Goose Point	Prespray	<i>Neotrypaea</i>	44	240 (8)	0 (0)
	1 mo post		58	7 (1)	0 (0)
	1 y post		49	114 (7)	6 (2)

Several transects were made across each bed, and observations were made within a 1-m^2 quadrat. Given are sample sizes (n) and means (± 1 SE).

issue are very similar to those made in terrestrial agriculture (Levins 1986, National Research Council 1996). There are also intriguing parallels between shrimp ecology and the ecology of terrestrial insects in agroecosystems (Metcalf 1986, Strong 1986). Although small-scale experimental studies such as this may continue to suggest hypotheses to be tested, only cooperative efforts between growers, agencies, and researchers will confirm results and provide practical long-term solutions that can be applied on a relevant production and ecosystem scale.

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EVIDENCE THAT QPX (QUAHOG PARASITE UNKNOWN) IS NOT PRESENT IN HATCHERY-PRODUCED HARD CLAM SEED

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ABSTRACT A protistan parasite known as QPX (Quahog Parasite Unknown) has been recently associated with disease and mortality of adult hard clams, *Mercenaria mercenaria*, from Canada to Virginia. There is concern that the organism may be transported in hatchery-reared seed. Tissue sections of 2,203 seed clams (<1–20 mm) from 13 different hatcheries in six states, collected from 1995 to 1997 and examined by pathologists in three laboratories, failed to show QPX or QPX-like organisms. Further, QPX was not detected in a total of 756 hatchery-produced clams examined during their first year of field growout. From this, we conclude that hatchery-produced seed clams are an unlikely source of QPX organisms.

KEY WORDS: hard clam seed, *Mercenaria mercenaria*, QPX, quahog, disease, parasite, hatchery

INTRODUCTION

A protistan parasite has been recently associated with disease and mortality of wild and cultured hard clams, *Mercenaria mercenaria* (Linnaeus, 1758), from Canada to Virginia (Whyte et al. 1994, Ragone Calvo et al. 1997, Smolowitz and Leavitt 1997, Smolowitz et al. in press). The parasite was first described in clams from the St. Lawrence River, Canada, in the late 1950s and early 1960s (Drinnan and Henderson 1963). It was subsequently found in juvenile and adult clams in a hatchery on Prince Edward Island, Canada, and at that time was given the acronym "QPX" for Quahog Parasite Unknown (Whyte et al. 1994). Morphologically similar organisms have since been found in clams from Massachusetts, New Jersey, and Virginia.

The proper classification of the QPX organism(s) is currently under investigation, and there may be more than one species involved. Whyte et al. (1994) pointed out similarities of the Canadian QPX to members of the Thraustochytriales and Labyrinthulales, which depending on the classification scheme, belong to the phylum Labyrinthomorpha (Pokorny 1985) or to the phylum Labyrinthulomycota (Porter 1990). Although members of these groups are common saprophytic organisms in marine and estuarine environments (Porter 1990), they have also been reported to cause disease in molluscs, especially those held in captivity (Polglase 1980, McLean and Porter 1982, Jones and O'Dor 1983, Bower 1987a).

In one reported disease outbreak, mortalities of up to 100% occurred in nursery-held juvenile abalones, *Haliotis kamtschatkana* (Jonas, 1845), that were heavily parasitized by a Labyrinthulid, *Labyrinthuloides haliotidis* (Bower 1987a). Subsequent investigations (Bower 1987b) showed that *L. haliotidis* could be transmitted directly from abalone to abalone by a flagellated zoospore stage of the parasite.

Hard clam culturists along the East Coast of the United States rely entirely on seed clams produced in hatcheries, which often ship seed to growers in distant regions of the coast. The finding of QPX-like organisms in cultured adult clams, combined with the possibility that they can be transmitted directly between clams, as is the case with *L. haliotidis*, has led to concern that the parasite might have been introduced via hatchery-produced seed and might be further spread in the same way.

Consequently, over the past 2 y, samples of seed clams from hatcheries in seven states (Maine, Massachusetts, New York, New Jersey, Virginia, North Carolina, and South Carolina) have been examined histologically for evidence of QPX, or QPX-like organisms, by our three laboratories. In an effort to provide up-to-date information to seed producers, growers, and resource managers, we present our combined findings in this report.

MATERIALS AND METHODS

Clam seed ranging in size from 1 to 25 mm (mostly <15 mm) shell length and generally no more than a few months old were brought directly to participating laboratories or shipped to them overnight. The smallest clams (1–5 mm) were placed directly into fixative (10% formalin in seawater or Davidson's fixative); the hinges were popped on clams from about 5 to 15 mm, and clams

TABLE 1.

List of hatchery-produced hard clam, *M. mercenaria*, seed samples examined histologically for QPX.

Collection Date	Hatchery Location	Hatchery Code	Shell Length (mm)	Number Examined	Diagnostic Laboratory
5/11/95	MA	A	5-8	50	LAAMP
11/9-27/95	MA	B	4-12	158	LAAMP
6/6/96	MA	A	5-6	150	LAAMP
6/13/96	NJ	C	11-16	23	LAAMP
6/21/96	ME → MA*	D	3-5	150	LAAMP
6/21/96	MA	E	≤2	75	LAAMP
7/24/96	NJ	C	5-8	150	LAAMP
7/24/96	MA	B	1-2	25	LAAMP
8/2/96	NJ → MA*	F	4-10	150	LAAMP
8/28/96	NJ	F	10-14	116	LAAMP
9/13/96	MA	A	15-20	50	LAAMP
10/16/96	MA	B	1-3	50	LAAMP
7/11/97	MA	A	3-5	50	LAAMP
8/28/97	MA	A	12-16	52	LAAMP
8/28/97	NJ	F	14-16	54	LAAMP
8/15/95	NJ	G	4-10	80	HSRL
10/19/95	NY	H	6-12	80	HSRL
10/22/95	NJ	G	6-12	240	HSRL
5/1/96	MA	I	2	100	HSRL
1/28/97	NY	J	5-14	50	HSRL
9/25/97	NC	K	7-9	50	HSRL
9/25/97	VA → NJ*	L	7-9	50	HSRL
10/6/97	NJ	C	10-13	50	HSRL
2/20/97	VA	M	<1	200	VIMS
Total				2,203	

Samples came directly from the hatchery/nursery, except for three samples (*) that were held briefly in nurseries in different states before sampling. Each hatchery was assigned a letter code (A-M) to differentiate among them. LAAMP, Laboratory for Aquatic Animal Medicine and Pathology; HSRL, Haskin Shellfish Research Laboratory; VIMS, Virginia Institute of Marine Science. None of the clams was diagnosed with QPX.

were placed into fixative. In both cases, the shells were allowed to decalcify in the fixative. Larger clams were shucked, and the meats were fixed. Clams 1-12 mm were embedded whole in paraffin; larger individuals were sectioned first. Tissue sections were mounted on slides, stained, and examined microscopically. A total of 2,203 seed clams directly from hatcheries were examined in this

manner between May 1995 and October 1997 (Table 1). Only two samples had been held in filtered (1-50 µm pore size) water before examination; the rest had been held in upwellers or raceways supplied with unfiltered seawater. An additional 756 hatchery-produced clams in their first year of field growout were examined in the same manner (Table 2).

TABLE 2.

List of hatchery-produced hard clam, *M. mercenaria*, seed samples examined for QPX during first year of field growout.

Collection Date	Hatchery Location	Growout Location	Growout Period (mo)	Shell Length (mm)	Number Examined	Diagnostic Laboratory
6/6&27/96	MA	MA	7	6-12	136	LAAMP
4/24/96	NY	NY	6	10-12	100	HSRL
4/12/96	NJ	NJ	6	6-8	120	HSRL
5/27/97	NJ	NJ	8	20-25	50	HSRL
5/15/96	VA	FL	<6	4-10	60	VIMS
10/24/96	ME	SC	<6	8-15	60	VIMS
11/20/96	VA	VA	9	5-15	60	VIMS
5/13/97	VA	SC	3	<5	60	VIMS
5/22/97	VA	VA	6	7-8	25	VIMS
5/22/97	VA	VA	5	9-13	25	VIMS
10/1/97	SC	FL	<6	12-16	60	VIMS
Total					756	

LAAMP, Laboratory for Aquatic Animal Medicine and Pathology; HSRL, Haskin Shellfish Research Laboratory; VIMS, Virginia Institute of Marine Science. QPX was not detected in any of the clams.

RESULTS AND DISCUSSION

No QPX-like organism was found in any of the 2,203 clams originating directly from hatcheries. We cannot discount the possibility that infection frequency was so low that we did not detect parasitized individuals in our samples or that our diagnostic methods missed some very light infections; however, the large number of clams that we examined makes this highly unlikely. Further, the scope of our investigation—encompassing 13 hatcheries in six states, seed clams of varying size and age, collections over 2 y, and examination by pathologists in three different laboratories—also lends support to the contention that hatcheries are not the source of QPX. In the late 1980s and early 1990s, six to eight samples of 50 seed clams each, from hatcheries in Massachusetts and New Jersey, were examined histologically without detection of any micro-organism resembling QPX (R. Hillman, Battelle Ocean Sciences, pers. comm. 1997).

The evidence that seed clams coming directly from hatcheries do not contain QPX-like organisms is further supported by histological examination of hatchery-produced clams diagnosed within a year of placement in field growout locations (Table 2). No QPX-like organisms were found in any of the 756 clams originating from hatcheries in six states and examined after periods ranging from 3 to 9 mo in the field. In fact, all findings of QPX-like organisms in hard clams under culture in the United States have been in adults, typically 1.5–2 y or older (Ragone Calvo et al. 1997, Smolowitz et al. in press). Smolowitz and Leavitt (1997)

monitored the acquisition of QPX infections in clams after they had been placed on infected leases and reported that none was detected until clams had been in the field for at least 1 y.

We believe that the most reasonable interpretation of the available data is that hard clams become parasitized with QPX-like organisms during field growout, not in hatcheries. Whether these organisms are facultative or opportunistic pathogens that invade clams already stressed by poor growing conditions is currently under investigation. Meanwhile, we hope that this report provides reassurance to growers, hatchery operators, and resource managers that hatchery seed is an unlikely source of QPX-like parasites in hard clams.

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ENHANCED GROWTH OF THE GIANT CLAM, *TRIDACNA DERASA* (RODING, 1798), CAN BE MAINTAINED BY REDUCING THE FREQUENCY OF AMMONIUM SUPPLEMENTS

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ABSTRACT Juvenile giant clams, *Tridacna derasa* (Roding, 1798), of 14–17 mm shell length were exposed to no (control), two, three, or five additions of 40 μ M ammonium sulfate per week in mass culture conditions for 45 days. Growth of clams, in terms of increase in wet weight and shell length, was significantly greater in the ammonium-enriched conditions compared with the control; however, no significant differences in growth occurred among juveniles exposed to ammonium for 2, 3, or 5 days/wk. Mean density of zooxanthellae per clam also increased significantly with increasing frequency of ammonium addition. The degree of fouling by epiphytic algae (*Boodlea* sp. and *Enteromorpha* sp.) was significantly greater in tanks receiving ammonium 5 days/wk than in tanks receiving ammonium twice per week. These results indicate that supplementing with ammonium sulfate 2 days/wk is sufficient to maintain high growth rates during land-based culture of juvenile *T. derasa*. The reduced frequency of these nutrient supplements also helps alleviate algal fouling in rearing tanks.

KEY WORDS: *Tridacna derasa*, nitrogen, ammonium, nutrition, aquaculture

INTRODUCTION

Giant clams (Tridacnidae) can be reared successfully throughout both the land-based nursery and ocean growout stages of production without the addition of food (Heslinga and Fitt 1987). These bivalve molluscs filter particulate matter from the water column using their gills, as well as receive photosynthates and other nutrients released by their symbiotic zooxanthellae (Streamer et al. 1988, Rees et al. 1993, Hawkins and Klumpp 1995). The zooxanthellae (i.e., dinoflagellate *Symbiodinium* spp.) live at high densities within the mantle (Norton et al. 1992) and provide the host with sufficient nutrients for growth and respiration (Fisher et al. 1985, Klumpp et al. 1992).

In aquaculture, giant clam larvae are usually “inoculated” with zooxanthellae from Days 8 to 15, depending on the species (Gervis et al. 1996), and are then transferred from the hatchery into outdoor nursery tanks. At ICLARM Coastal Aquaculture Centre, Solomon Islands, juveniles remain in nursery tanks until they reach 25 mm shell length (SL) before being sold to growers. To enhance the growth rates of giant clams throughout this period, nitrogen (N), usually in the form of an ammonium salt, is added daily to the nursery tanks (Braley 1992). Increased growth occurs because both the giant clams and their zooxanthellae can fix dissolved inorganic nitrogen (Fitt et al. 1993, Rees et al. 1994), which is incorporated into the tissues (Belda et al. 1993). For example, the addition of N has increased growth of clams of 28 mm SL by 375% (Hastie et al. 1992), compared with nonenriched clams.

Until recently, enriching production tanks with up to 50 μ M N each day was thought to optimize the growth of juvenile giant clams (Heslinga and Fitt 1987, Hastie et al. 1988, Fitt et al. 1993). However, it is now evident that the response of *Tridacna maxima* (Roding, 1798) to increased N is dependent on the size of juveniles. Grice and Bell (in press) found that individuals of 5 mm SL did not benefit from N > 10 μ M. As the juveniles grew, however, they used increased quantities of N, and individuals of 18 mm SL grew best at concentrations of 80 μ M. Thus, the benefits of adding

nutrients during the culture of *T. maxima* are dependent on the size of juveniles.

Despite the benefits of adding N to nursery tanks, 50% of the nitrogen is lost to other organisms or is flushed out with the wastewater (Fitt et al. 1993). Previous research has also shown that giant clams maintained in elevated ammonium conditions for prolonged periods assimilate only a small percentage of the N (Wilkerson and Trench 1980, Rees et al. 1994). Excess nitrogen allows other organisms, particularly “nuisance” algae, to proliferate in nursery tanks. Algae can smother juvenile giant clams and reduce light availability for zooxanthellae photosynthesis. Unless the growth of fouling algae is controlled by herbivorous grazers and manual removal, it causes high levels of mortality of juvenile giant clams (Braley 1992, Braley et al. 1992, Fitt et al. 1993). The aim of this study was to determine whether it is possible to maintain enhanced growth of clams, but limit the growth of algae, by reducing the frequency of N enrichment.

METHODS

Tridacna derasa juveniles of 14–17 mm SL, and enrichment of 40 μ M ammonium sulfate, were used to test the null hypothesis that frequency of ammonium supplementation did not affect the growth of juveniles in nursery tanks. Clams from the same cohort were harvested from 5,000-L, land-based production tanks at the ICLARM Coastal Aquaculture Centre, Solomon Islands, and were stocked into 12 150-L concrete tanks at a density of 300 individuals per tank. After the clams had been left to settle for 2 days, three tanks were allocated at random to four levels of frequency of ammonium supplementation: 0 (control), 2, 3, and 5 days of N enrichment per week.

Individuals in nutrient-enriched tanks were supplied with spikes of dissolved ammonium sulfate at 10:00 h on appropriate “feeding” days to elevate N concentrations in the tanks to 40 μ M. Concentrations of N in the control tanks were consistently <1 μ M. Water flow was turned off to all tanks for the first hour after the addition of nutrients. The experiment continued for 45 days.

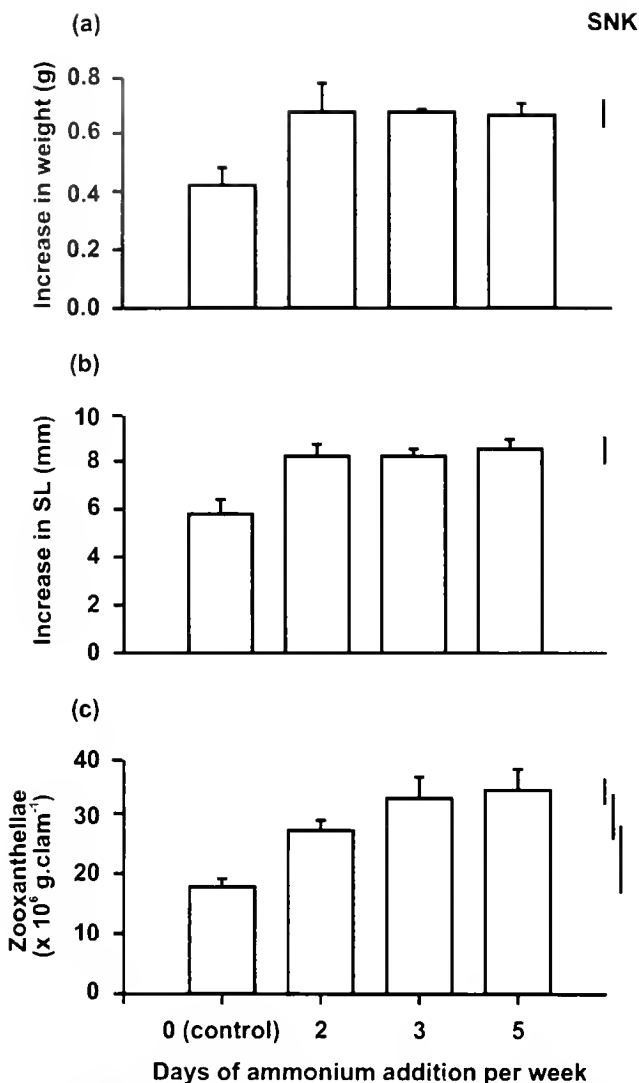


Figure 1. Mean ($n = 3$) growth responses of *T. derasa* in terms of (a) increase in wet weight, (b) increase in SL, and (c) density of zooxanthellae to the different frequencies of 40 μ M ammonium addition after 45 days. Error bars represent standard errors. Vertical line links mean that growth does not differ significantly by the SNK test.

The experimental tanks were located outdoors, covered with 50% shademesh, supplied with 200-mL unfiltered seawater per min pumped from the adjacent coast, and aerated for 16 h/day. Thirty herbivorous gastropods (*Cerithium* sp., 10–25 mm) were placed into each tank on the seventh day of the experiment to assist in controlling the growth of fouling algae. Detritus and algae were removed from tanks on a weekly basis.

Growth was estimated as increase in mean wet weight (g), and SL (mm), over the experimental period. To obtain these estimates, measurements were taken from 50 individuals selected at random from each tank at the beginning and end of the experiment. We measured wet weight to the nearest mg and SL to the nearest 0.1 mm. There were no significant differences in mean sizes of clams for each level of the frequency treatment at the start of the experiment (one-way analysis of variance [ANOVA] wet weight: $F_{3,8} = 1.5$, $p = 0.68$; SL: $F_{3,8} = 0.51$, $p = 0.28$).

Mean density of zooxanthellae was also estimated for each level of the frequency treatment at the end of the experiment. Flesh

from three clams, selected at random from each tank, was extracted and blended in 10 mL of filtered seawater until homogenized. The zooxanthellae in each homogenate were counted with a hemocytometer to estimate the density of zooxanthellae per g of clam in each tank.

At the end of the experiment, there were differences in algal fouling among levels of the nutrient frequency treatment; tanks exposed to 5 days of N addition per week appeared to have the most algae, although the differences in algal fouling were not quantified.

To assess the "costs" of nutrient addition, in terms of measures needed to control algae, we set up a second experiment to investigate the effect of N supplementation frequency on algal fouling. In this experiment, stocking density of clams and levels of nutrient frequency were the same as in the initial experiment. Thirty herbivorous grazers (*Cerithium* sp.) were added to each tank after 7 days, as in the first experiment; however, tanks were not cleaned manually. After 60 days, mean algal cover was measured in each tank using a quadrat of 0.01 m². The quadrat was thrown haphazardly to five different locations within each tank. Algae inside the quadrat were then removed from the base of the tank with a scalpel blade and siphoned onto a 53- μ m nylon mesh sieve. The algal samples were rinsed, sun dried, and weighed to the nearest mg.

One-way ANOVA was used to determine the effects of N enrichment frequency on the mean increase in wet weight and SL of giant clams, as well as the quantity of zooxanthellae per clam, at the end of the experiment. A nested one-way ANOVA, with tanks nested in levels of N addition, was used to analyze variation in algal biomass in the second experiment. For each analysis, data were checked for homogeneity of variance and transformed to \log_{10x} to meet this assumption where necessary. Where significant differences occurred among means, the Student-Newman-Keuls (SNK) test was used to identify the nature of these differences.

RESULTS

Experiment 1—Growth Responses of *Tridacna derasa*

The addition of ammonium sulfate for 2, 3, and 5 days caused a significant increase in mean wet weight ($F_{3,8} = 6.34$; $p < 0.05$) and mean SL ($F_{3,8} = 10.70$; $p < 0.004$) compared with values in control tanks. There were, however, no significant differences in

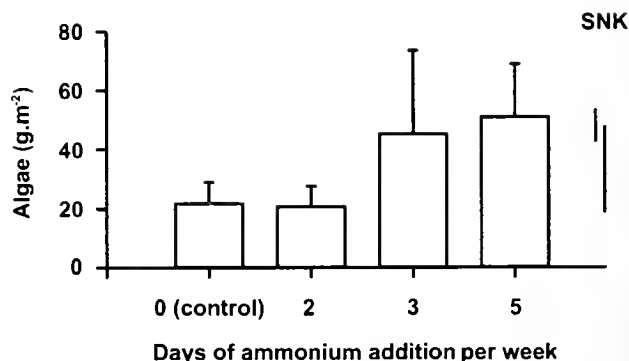


Figure 2. Mean ($n = 15$) biomass of algae colonizing the bases of the tanks after 60 days of exposure to different frequencies of 40 μ M ammonium addition. Error bars represent standard errors. Vertical line links mean that biomass does not differ significantly by the SNK test.

the increase in wet weight and SL among clams exposed to ammonium enrichment for 2, 3, and 5 days/wk (Fig. 1 a and b).

In contrast, the mean density of zooxanthellae per clam was significantly affected by frequency of nutrient enrichment ($F_{3,8} = 7.20$; $p < 0.005$). In general, zooxanthellae increased concomitantly with the frequency of ammonium addition, and the density of zooxanthellae in tanks receiving 40 μM N 3 and 5 days/wk was twice that of clams in control tanks (Fig. 1c).

Experiment 2—Algal Fouling

The two major species of algae colonizing tanks during this experiment were *Boodlea* sp. and *Enteromorpha* sp. The frequency of ammonium enrichment had a significant effect on the total biomass of the algae ($F_{3,8} = 4.2$; $p < 0.01$). Although the level of fouling in tanks receiving nutrients 3 and 5 days/wk was two to three times greater than that for control tanks and those supplemented 2 days/wk (Fig. 2), the SNK test was unable to discriminate logically among the means (Fig. 2). That is, the amount of algae in tanks receiving N 5 days/wk was significantly greater than that in control tanks and those supplemented 2 days/wk, but fouling of control tanks and those supplied with 2 and 3 days/wk was not significantly different.

DISCUSSION

This study shows clearly that N enrichment augmented the growth rate of giant clams, but that growth was not improved by increasing the frequency of nutrient supplements. In particular, the current practice of adding ammonium to giant clam nursery tanks

5 days/wk (e.g., Braley et al. 1992) was no more beneficial than additions twice per week.

Conversely, the density of zooxanthellae was higher in clams exposed to ammonium enrichment 3 and 5 days/wk than in those receiving enrichment for only 2 days/wk. This indicates that zooxanthellae benefited from more frequent exposure to elevated levels of N, but that the additional nutrients were not passed on to the host. A possible explanation for this result is that the clams used the majority of N for growth when it was available infrequently, and zooxanthellae only received "excess" nutrients for growth and reproduction when N was available more often. As the role of zooxanthellae increases in nutritional importance to giant clams as the clams mature (Fisher et al. 1985; Klumpp et al. 1992), our results suggest that the reliance of *T. derasa* of 14–17 mm SL on zooxanthellae for nutrients is not great enough to be affected significantly by the differences in the density of the symbionts.

There are at least three advantages in applying N for 2 days instead of 5 days/wk. First, the cost of fertilizer can be reduced by 60%. Second, fouling by epiphytic algae can be reduced, thus providing better growing conditions for clams (cf. Fitt et al. 1993). Third, the effluent from giant clam nurseries will contain less nitrogen and have a lower effect on the environment.

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THE SCALLOP *PECTEN ZICZAC* (LINNAEUS, 1758) FISHERY IN BRAZIL

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ABSTRACT In this article, we summarize data available on a previously unreported and very intensive fishery that targetted the tropical scallop *Pecten ziczac* on the southern Brazilian shelf during the 1970s and early 1980s. Scallop beds were found between 24°26'S and 26°30'S, on sandy substrates between 30 and 50 m deep, on the inner continental shelf. Brazilian fishery of *P. ziczac* began in 1972, when trawlers licensed for the industrial pink-shrimp fishery (*Penaeus paulensis* and *Penaeus brasiliensis*) modified their shrimp otter-trawl to catch scallops. Landings of *P. ziczac* rose from 4.5 tons in 1972 (first year of production) to 3,799 tons in 1975 and were followed by a pronounced decline in subsequent years, with a minimum of 8.7 tons in 1978. A second and higher peak in scallop production started in 1979, with a total of 8,845 tons recorded in 1980. However, after the 1980 peak, landings of *P. ziczac* were drastically reduced, and scallops returned to the initial condition of a minor by-catch item in the shrimp fishery. Our recent surveys point to a complete collapse of the resource. Since its beginning, the scallop fishery in Brazil was conducted without any specific scientific monitoring and legal regulation.

KEY WORDS: scallop, *Pecten ziczac*, fishery, Brazil

INTRODUCTION

Over the past two decades, scallops have constituted an important shellfish resource for some Latin American countries. This has been the case for *Pecten vogdesi* (Arnold, 1906), *Argopecten circularis* (Sowerby, 1835), and *Lyropecten subnodulosus* (Sowerby, 1835) in Mexico (Felix-Pico 1991); *Pecten papyraceus* (Gabb, 1873) in Venezuela (Salaya and Penchaszadeh 1979); *Chlamys theuelcha* (d'Orbigny, 1846) in Argentina (Orensanz et al. 1991); and *Argopecten purpurata* (Lamarck, 1819) and *Chlamys lischkei* (Dunker, 1850) in Chile (Piquimil et al. 1991).

Many of these fisheries are well known, and their respective species have been intensively researched. In a recent review on scallop ecology, Brand (1991) stated that *C. theuelcha* is the only species harvested in the southwestern Atlantic. In this article, we summarize the data available on a previously unreported and very intensive fishery that targetted the tropical scallop *Pecten ziczac* on the southern Brazilian shelf during the 1970s and early 1980s.

Although scallop production in Brazil has been one of the highest compared with that in other Latin American countries, no studies have been conducted on the biology and fishery management of the species, except for some reports on distribution (see below) and industrial processing (Morais and Kai 1980). Most of the available information about the biology of *P. ziczac* has been produced in Venezuela, where its culture has been developed (e.g., Lodeiros et al. 1992, Freitas et al. 1993a, Freitas et al. 1993b, Freitas et al. 1995, Lodeiros and Himmelman 1994, Lodeiros and Himmelman, 1996). This review is part of an extensive research program currently developed by FACIMAR and CEM, with the aim of studying aspects of the population dynamics, fishery biology, and stock assessment of *P. ziczac* in southern Brazil.

MATERIALS AND METHODS

This article is based on data retrieved from reports and bulletins produced by the fishery management agencies (Brazilian Institute for the Environment and Renewable Resources–IBAMA and Fishery Institute of São Paulo–IP/SP) and, in the case of unpublished information, from archival sources and manuscripts. We also had access to valuable material from the Brazilian agencies involved with international trade (Brazilian External Commerce Department–Secretaria de Comércio Exterior, Ministério da Indústria, do Comércio e do Turismo, Brasília–DF) and food quality (Brazilian Service of Food Inspection–Secretaria de Inspeção de Produto Animal–SIPA, Ministério da Agricultura, Brasília–DF), which were used to assess the destination and economic importance of the resource, as well as to gauge the reliability of other sources of information. Most of these reports and data were only locally available, and therefore, Brazil has been excluded from the picture of world scallop production.

Unfortunately, we could not recover data from vessel logbooks, and because more specific data on the fishery have not been published previously, it was not possible to calculate fishing effort and scallop yield. In addition, most of the old statistics and reports were dispersed, making it difficult or even impossible to complete some series of data.

RESULTS

Distribution of P. ziczac Beds

The distribution of the scallop beds in southern Brazil is known from surveys conducted by the R/V Riobaldo and R/V Diadorim in 1974 and 1975 (Jones et al. 1974, Sachet et al. 1974, Zenger et al. 1974, Zenger et al. 1975, Agnes et al. 1975, Agnes and Jorge 1975,

Agnes and Zenger 1975). Scallops were found between 24°26'S and 26°30'S, with the most important concentrations occurring between 24°55'S and 26°20'S, in regions between south-southeast of Bom Abrigo Island (São Paulo State) and east of Paranaguá Bay (Paraná State) (Fig. 1). The beds occurred only on sandy substrates between 30 and 50 m deep, and no scallops were found on muddy bottoms.

Fleet Characteristics

Since the beginning of the Brazilian fishery, scallops have been caught mainly by trawlers licensed for the industrial pink-shrimp (*Penaeus paulensis* Perez Farfante, 1967 and *Penaeus brasiliensis* Latreille, 1870) fishery. The latter experienced rapid growth during the 1960s and early 1970s, when the Brazilian government

implemented actions to increase the activity (Iwai 1973). As a direct consequence, the number of vessels increased and their efficiency improved.

Until 1969, these vessels operated a single otter-trawl using the side-trawl system. Between 1969 and 1972, vessels were converted to the 45% more efficient double-trawling method developed in the United States (Valentini et al. 1991). In this system, two otter-trawls with 12- to 20-m-long ground and otter ropes are operated simultaneously, one from each side of the vessel, at speeds ranging from 3.5 to 4.0 knots (Iwai 1973). Most of the boats were wooden hull and powered by engines with 250–350 horsepower with a crew of five or six (Iwai 1973). Mean vessel length was 19.5 m (Valentini et al. 1991).

Anecdotal information provided by some captains gave notice that some types of dredges and beam-trawls were tried but soon

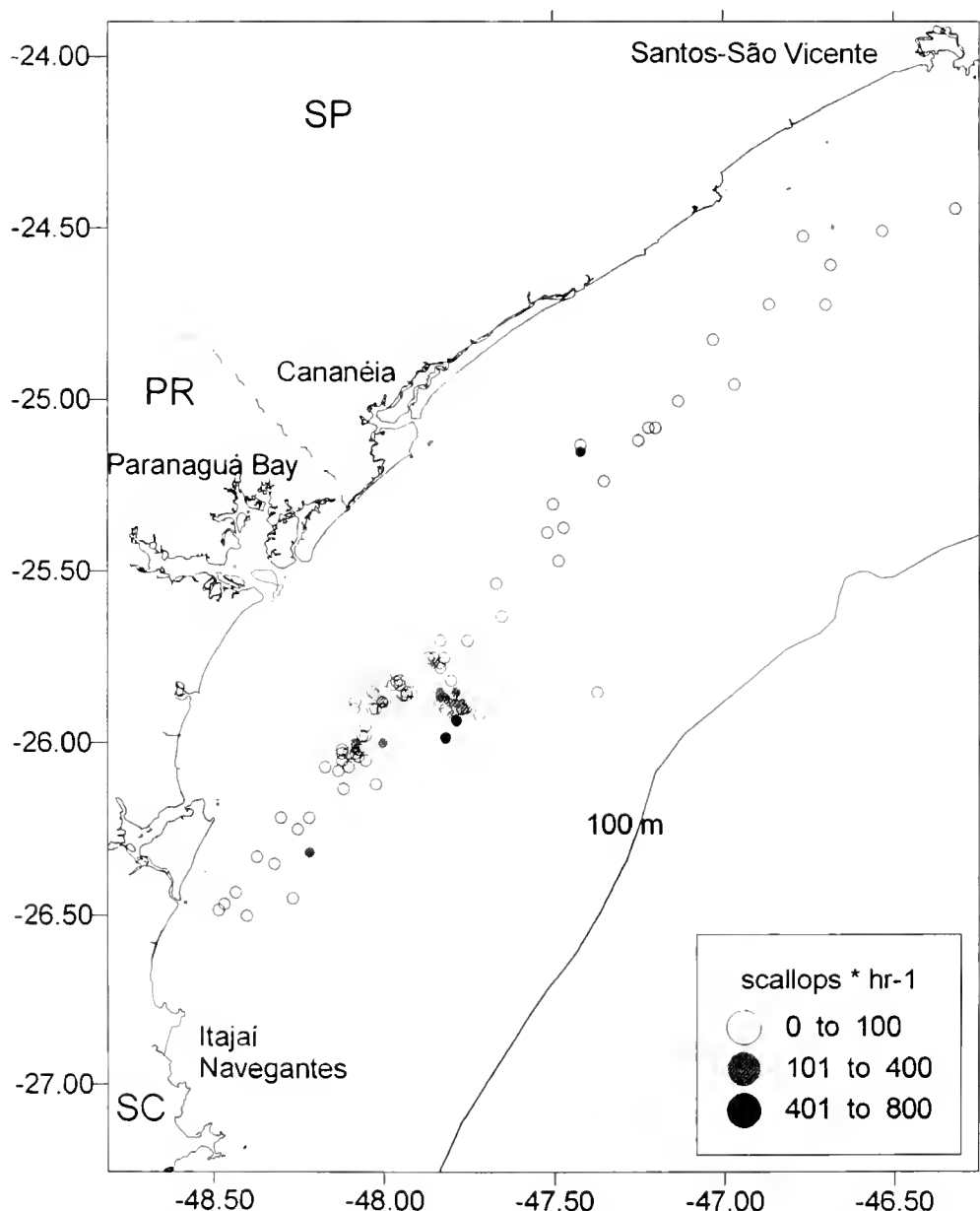


Figure 1. Area of distribution of *P. ziczac* beds in 1974 and 1975, based on scallop catch rates obtained during surveys conducted by R/V Diadorim and R/V Riobaldo, and main ports of landing.

abandoned. Therefore, during the period of highest production of scallops, fishermen used the same type of shrimp otter-trawl to catch them, although with a few modifications. These modifications were heavier tickler chains and nets reinforced by the use of more resistant threads and bottom protection meshes (Rebello Neto 1980). Fishing was conducted mainly between 6 p.m. and 6 a.m., with the best yields occurring between 10 p.m. and 4 a.m.

Although the exact number of vessels involved in the scallop fishery remains unknown, shrimp trawlers registered in São Paulo and Santa Catarina (states that concentrated the landings) between 1973 and 1982 ranged between 115 and 189 units (MINISTÉRIO DA AGRICULTURA 1985b). According to Rebello Neto (1980), the number of vessels landing scallops in Santa Catarina ports between April and July 1979 varied monthly between 29 and 76. Considering that this state accounted for 45% of the scallop landings, it is probable that most of the registered shrimp trawlers had fished for *P. ziczac* during the period of major production.

The Fishery

The Brazilian fishery for *P. ziczac* began in 1972, when the first landing was recorded in São Paulo. Before that year, scallops were caught as by-catch in the shrimp fishery and discarded or used for local consumption only. Targetting of scallops by the fleet likely resulted from three main factors: (1) in 1972, there was a rapid increase in the quantities of scallops caught by vessels operating from São Paulo (Rebello Neto 1980); (2) at that time, the number of vessels fishing for pink-shrimp from São Paulo and Santa Catarina harbors increased from 58 in 1966 to 225 in 1972 (MINISTÉRIO DA AGRICULTURA 1985b), and consequently, shrimp catch per unit of effort (CPUE) dropped from ca. 25 kg h⁻¹ in 1965 to ca. 5 kg h⁻¹ in 1973 (Fig. 2); and (3) there was a great demand for scallops in the North American market and an increase in the international prices due to the decline in the catches from the Georges Bank fishery (United States and Canada) (Orensanz et al. 1991, Shirley and Krus, 1995). Therefore, the landings of *P. ziczac* rose from 4.5 tons in 1972 (first year of production) to 3,799 tons in 1975 (Fig. 2) and were followed by a pronounced decline in subsequent years, with a minimum of 8.7 tons in 1978. A second

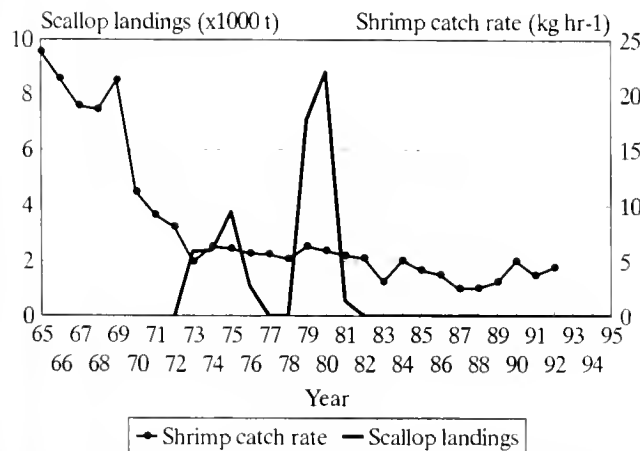


Figure 2. *P. ziczac* annual landings and pink-shrimp (*P. paulensis* and *P. brasiliensis*) catch rates (kg h⁻¹) in southern Brazil between 1965 and 1995 (data for scallops after 1988 corresponded only to Santa Catarina landings).

and higher peak of scallop production started in 1979, with a total of 8,845 tons recorded in 1980. During the years of major production (1975 and 1980), landings of scallops exceeded many times the catches of shrimp by the São Paulo and Santa Catarina fleets during the same periods (2,295 tons in 1975 and 1,537 tons in 1980) (MINISTÉRIO DA AGRICULTURA 1985b). In contrast to the huge production of 1972–1975 and 1979–1980, no more than 10–20 tons were caught per year after 1981. Since 1988, the fishing authority of the state of São Paulo (Instituto de Pesca) has no longer recorded *P. ziczac* landings in the state.

Until 1995, harvesting of scallops added up to 26,221 tons. São Paulo (SP) accounted for 55% of landings, and Santa Catarina (SC) accounted for the remaining 45%. The main ports were Santos (SP) with 11,836 tons landed (45%), São Vicente (SP) with 2,550 tons (9.7%, active only between 1973 and 1976), Cananéia (SP) with 56 tons (0.3%), and Itajaí and Navegantes (SC), with a total landing of 11,790 tons (45%). At least two other ports had some importance during shorter periods São Sebastião (northern of SP) and Rio Grande (southern of Rio Grande do Sul State), but their contribution is unknown.

It is difficult to assess if there was a real seasonality in scallop production, because monthly landings were extremely variable from year to year. However, there was a tendency for the highest values of harvesting to occur in the austral summer and winter months (Fig. 3). On the other hand, considering the entire period over which the fishery operated, there seems to be a cycle of 3–5 y in scallop production, the causes of which are not yet understood (Fig. 4).

There are no temporal series of effort or CPUE data for the scallop fishery from Brazil. However, a short-term estimation conducted by Rebello Neto (1980) revealed that between April and July 1979, the production per vessel in Santa Catarina averaged 12 tons, with a mean CPUE of 243 kg h⁻¹ in 3 or 4 nights of trawling. In addition, catches of up to 30 tons per vessel were not uncommon during the best years of production.

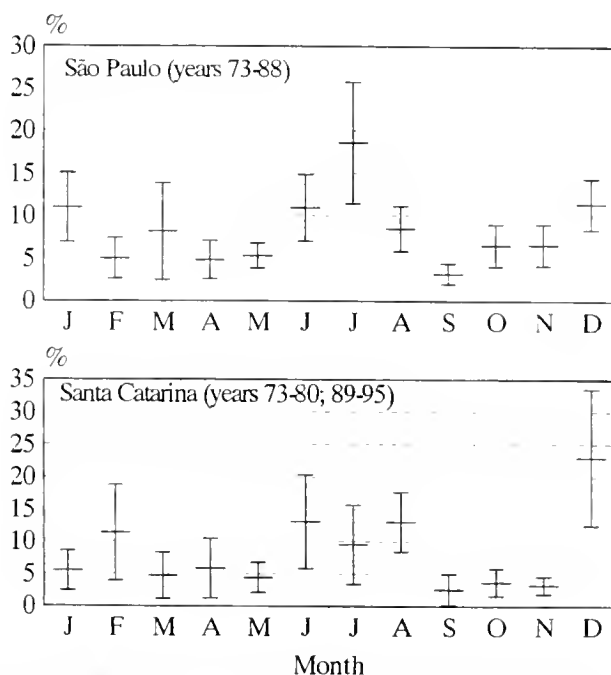


Figure 3. Mean percent contribution ($\pm 95\%$ confidence interval) of each month to total *P. ziczac* production.

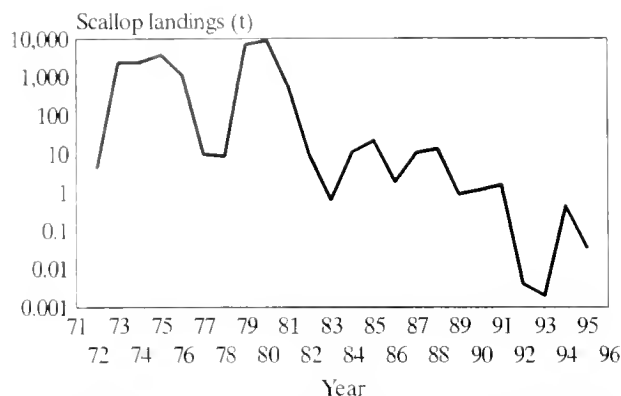


Figure 4. *P. ziczac* annual landings in southern Brazil, showing a 3- to 5-y cycle of production (data after 1988 corresponded only to Santa Catarina landings).

Manufacture and Destination of the Production

Unlike in many countries where scallops are shucked aboard (Felix-Pico 1991; Shirley and Kruse 1995), in Brazil, they were landed "in natura" and processed in plants installed in the port cities or in their neighborhoods. The production was completely destined for the international market, mainly as frozen muscle. Fishery statistics and data on scallops processed for exportation (MINISTÉRIO DA AGRICULTURA 1980, 1981, 1982, 1984, 1986A) show the same pattern of temporal change in scallop production, although with very different values in some years (Table 1). According to fishery statistics, the year of highest landing was 1980 with 8,845 tons, whereas the peak in scallop production occurred in 1979, when 11,997 tons were processed in plants subjected to fiscal control of SIPA. This represents a difference of 35.6% between the two estimates of maximum annual quantities of scallop production. Between 1980 and 1986, processing of scallops was similar for São Paulo (4,814 tons, 42.3%) and Santa Catarina (6,465 t; 56.9%). Rio Grande do Sul accounted for the remaining 0.8%, processing 88 tons in 1980.

Statistics for scallop meat exports follow the cycles of fishery production and processing with two major periods of sales (Table 1). The price per kilogram of muscle increased continuously, changing from US\$ 2.73 kg⁻¹ in 1973 to US\$ 6.55 kg⁻¹ in 1981. Between these years, scallop sales generated an export value of US\$ 6,057,033.00. However, we suspect that the export data, mainly from 1979 on, were underestimated. In *P. ziczac*, the muscle represents nearly 20% of its total wet weight (Morais and Kai 1980, Pezzuto unpubl.). Applying a five-fold conversion factor to the data of muscle exports, it was possible to notice that the values reported for the first period of production (1973–1978) were close to the values showed by fishery and processing statistics. However, in 1979, the estimated exports of whole scallops (1,649.9 tons) corresponded to only 23% of the 7,086 tons (fishery statistics) or to 13.7% of the 11,997 tons (SIPA statistics) produced in the same period. We have no information about this high rate of discarding in ports or processing plants, and it is impossible that the Brazilian domestic market could have absorbed the excess of production.

France and the United States were the main importers of scallops from Brazil, accounting for 93% from the total exports (Table 2). The Netherlands, Belgium, and Argentina were of lesser importance.

DISCUSSION

Scallop harvesting in Brazil was one of the most significant as compared with that in other Latin American scallop fisheries. Although peaks in production of *P. ziczac* reached at least 3,799 (1975) and 8,845 (1980) tons (according to fishery statistics), peak annual landings in other countries were 4,601 tons in Mexico (Felix-Pico 1991), 1,720 tons in Venezuela (Salaya and Penschazadeh 1979), ca. 4,500 tons in Argentina (Orensanz et al. 1991), and 5,278 tons in Chile (Piquimil et al. 1991).

However, after the maximum landings of 1980 the production of *P. ziczac* became insignificant. Scallops returned to the initial condition of a minor by-catch item in the shrimp fishery, being

TABLE 1.
P. ziczac statistics.

Year	Fishery Production (tons)	Industry Processing (tons)	Exportation			
			Muscle (kg)	US\$ kg ⁻¹	US\$ Total	Scallops (t*)
1972	4.5	—	—	—	—	—
1973	2,356.3	266	156,950	2.73	428,951.00	784.7
1974	2,402.7	1,702	270,188	2.90	784,175.00	1,350.9
1975	3,799.0	2,512	432,254	3.96	1,712,831.00	2,161.3
1976	1,095.4	505	108,850	4.25	462,878.00	544.2
1977	9.7	7	9,240	1.79	16,553.00	46.2
1978	8.7	205	5	5.60	28.00	0.02
1979	7,086.3	11,997	329,779	6.09	2,009,602.00	1,648.9
1980	8,845.3	9,962	98,748	5.79	571,742.00	493.7
1981	552.1	1,283	10,918	6.55	71,551.00	54.6
1982	8.9	15	—	—	—	—
1983	0.6	3	—	—	—	—
1984	11.1	4	—	—	—	—
1985	21.6	80	—	—	—	—
1986	1.9	22	—	—	—	—

Fishery production and industry processing values correspond to fresh whole scallops. Scallops (t*) were estimates of whole scallops obtained from a five-fold conversion factor applied to exported muscle weight. For data sources, see text.

TABLE 2.

Data from importers of Brazilian scallop (*P. ziczac*) muscle between 1973 and 1981.

Country	Muscle (kg)	US\$
United States	823,992 (58%)	3,565,664.00
France	503,653 (36%)	2,147,689.00
Netherlands	71,207 (5%)	290,631.00
Belgium	8,835 (1%)	37,746.00
Argentina	8,740 (1%)	15,303.00
Total	1,416,422 (100%)	6,057,033.00

For data sources, see text.

caught only during short periods when the areas of shrimp distribution coincided with the scallop beds during the year (Perez and Pezzuto unpubl.). Research cruises conducted since December 1995 have shown extremely low densities of *P. ziczac* in the remaining beds (Borzone and Pezzuto 1997, Pezzuto and Borzone 1997), pointing to a complete collapse of the resource. The best yields obtained during the cruises were only 35 individuals per hour, using a heavy 2-m-wide beam-trawl at trawling speeds of 3–4 knots.

Since its beginning, the scallop fishery of Brazil was conducted without any specific monitoring and/or regulation. Management has concentrated on the resources targetted by the fleet (shrimps),

while by-catch species received little if any attention. Using the *P. ziczac* fishery as a dramatic case study, we suggest that much more effort must be directed to the study of nontraditional Brazilian shellfish resources, including the improvement of statistical data collection, storing, and distribution.

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MOVEMENTS AND HABITAT USE OF THE QUEEN SCALLOP, *EQUICHLAMYS BIFRONS*, IN THE D'ENTRECASTEAUX CHANNEL AND HUON RIVER ESTUARY, TASMANIA

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ABSTRACT Movements and habitat use of the queen scallop, *Equichlamys bifrons*, were investigated in the D'Entrecasteaux Channel and Huon River estuary, Tasmania. Movements in *E. bifrons* were very local. In areas of strong current flow, movement occurred predominantly along the axis of the water current. There was evidence for an up-slope movement in areas of weak current flow. In sheltered bays, *E. bifrons* occurs more frequently in seagrass beds than on adjacent sand areas. Possible reasons for this distribution were investigated with both tethered and tagged scallops. Predation by the 11-armed spiny starfish, *Coscinasterias muricata*, and the octopus, *Octopus maorum*, on scallops was much higher on sand than in seagrass. Tagged scallops moved from sand areas to seagrass areas, but not from seagrass to sand.

KEY WORDS: Scallop, *Equichlamys*, habitat, movement

INTRODUCTION

Equichlamys bifrons (Lamarck 1819) (queen scallop) is a large scallop (150 mm widest diameter) found in New South Wales, Victoria, South Australia, and Tasmania (MacPherson and Gabriel 1962). In the D'Entrecasteaux Channel and Huon River estuary, Tasmania, *E. bifrons* occurs with the doughboy scallop (*Chlamys asperimus*) and the commercial scallop (*Pecten fumatus*). All three species have been fished in this area intermittently since about 1915.

The commercial importance of scallops has resulted in their biology being the subject of much scientific research. Despite this, the habitat requirements of scallops are poorly understood. This is especially true for *E. bifrons*. The distribution of *E. bifrons* in the D'Entrecasteaux Channel was described briefly by Olsen (1955), who observed that it occupies a shallow range (2–14 m) and often occurs in association with the seagrass *Heterozostera tasmanica* at a depth of 2 m, in close proximity to rocky reefs. He also noted that adult *E. bifrons* are relatively sedentary and lie in large, saucer-shaped depressions, but juveniles are vigorous swimmers.

The aims of this study were to investigate movement in *E. bifrons* to determine what factors might influence the direction of movement and to see over what distance movement occurs. During preliminary observations, we observed that in shallow bays, *E. bifrons* occurred more frequently in seagrass beds than on adjacent sand patches. The possible effect of predation on this distribution was investigated.

MATERIALS AND METHODS

Study Sites

E. bifrons was studied at one site in the D'Entrecasteaux Channel (Middleton) and two sites in the Huon River estuary (Eggs and Bacon Bay and Deep Bay) (Fig. 1). The scallop beds at Middleton occur at a depth of 14 m, on a bottom of coarse sand and broken shell. Strong tidal currents (0.5–1.0 m sec⁻¹) flow along the north-south axis.

In Eggs and Bacon Bay and Deep Bay, *E. bifrons* is found in depths of between 2 and 4 m, on a silty bottom scattered through-

out the seagrasses *H. tasmanica* and *Halophila australis*. These sites are sheltered and subject to weak currents.

Movements

Movements of scallops were studied at Middleton and Deep Bay. Scallops (31–126 mm shell height) were collected by SCUBA divers and taken to the surface, where they were placed into large bins containing seawater. "Hallprint" flexible plastic tags (4 × 9 mm) were attached flush to the lower valve of *E. bifrons* with cyanoacrylate adhesive. Divers placed the tagged scallops within a circle of 3-m radius of an anchored buoy at each site. Tagged scallops at Middleton were placed on coarse sand and shell fragment substrate. Tagged scallops at Deep Bay were placed in the seagrass bed. Tagged scallops were put in place between September 16 and October 29, 1992, at Middleton (n = 373), and on October 3, 1992, at Deep Bay (n = 89).

The dispersal of tagged scallops was determined on April 18 and 20, 1993, for Middleton and Deep Bay, respectively. Transect lines were placed north-south and east-west by divers so that they intersected at the anchored buoy and extended out to a radius of 30 m. Directions in this study were measured in relation to magnetic north. A peg was pushed into the ground next to the anchored buoy, and a rope marked in 2-m increments was attached to the peg. Divers swam concentric circles on the rope and recorded the position of scallops relative to the transect lines and using compass bearings, within 2-m bands and extending out to a 30-m radius.

The axis along which the current flows was determined at each site by pushing a peg into the sea floor with an attached piece of neutrally buoyant cord. The direction in which the cord pointed was measured with a compass. This was repeated in all stages of the tide cycle during 30 dives at each site.

The distribution of the scallops was analyzed using circular statistics (Batschelet 1981, Zar 1984). Circular data were grouped into 30° intervals, and the mean angle (\bar{a}) of movement and 95% confidence interval were calculated. Because a randomly distributed circular sample can still display a calculable mean (Zar 1984), the Rayleigh test was used to test whether there was statistical evidence of directional movement, i.e., to determine that a valid mean angle of movement existed. The *V* test, which is a modifi-

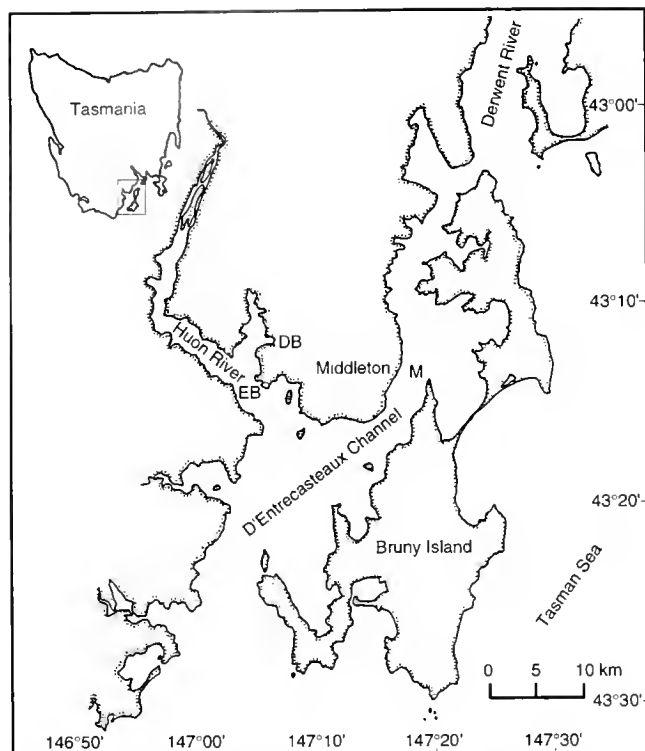


Figure 1. D'Entrecasteaux Channel and Huon River estuary, showing study sites. M = Middleton; EB = Eggs and Bacon Bay; DB = Deep Bay.

cation of the Rayleigh test, was used to test the hypothesis that the dispersal of the scallops at Middleton was along the axis of the current. The null hypothesis was that the population was uniformly distributed. The *V* test was not applied to the Deep Bay results because there was no obvious axis of current flow.

Predation

At Deep Bay and Eggs and Bacon Bay during 30 h of diving, divided approximately equally between the seagrass and the sand habitats, about 500 *E. bifrons* individuals were observed in seagrass beds as opposed to 6 individuals on surrounding sand patches. A preliminary tethering experiment was conducted at Eggs and Bacon Bay to investigate the importance of predation in determining this distribution. This site was chosen because of the clear demarcation between sand and seagrass areas.

On January 14, 1993, scallops (90–100 mm shell height) were tethered to compare survival rates. Scallops were tethered and spaced evenly along two 5-m lengths of white leadcore rope held close to the bottom by weights. These ropes were laid in parallel, 5 m apart, one in seagrass, and the other on the sand substrate. Scallops were tethered by 20-cm loops of 3.6-kg test monofilament threaded through small (11 × 7 mm) flexible plastic tags glued flush to the auricle of the shells. Seventeen scallops were tethered in seagrass, and 19 were tethered on sand. The numbers of tethered scallops alive in each location were monitored at 5, 7, 16, and 28 days from the start of the experiment.

Because tethering may have increased predation on scallops by restricting their movement, a separate experiment was set up on February 27, 1993, that allowed the scallops unrestricted movement. Fifty-four scallops (90–100 mm shell height) were collected

and individually tagged using techniques described for the movement studies. Half of the tagged scallops were placed in seagrass, and the other half were placed on the adjacent sand (Fig. 2). Scallops were placed next to small numbered net floats anchored on a short length of rope. On April 17, 1993, the area was thoroughly searched by divers and the position of live and dead tagged scallops was recorded. The condition of the shells of dead scallops was recorded to determine how they had died.

RESULTS

Movements

The axis of current flow at Middleton was 010°. At Deep Bay, the axis of current flow was variable and very weak.

In the dispersal surveys, 182 (49%) scallops were found alive

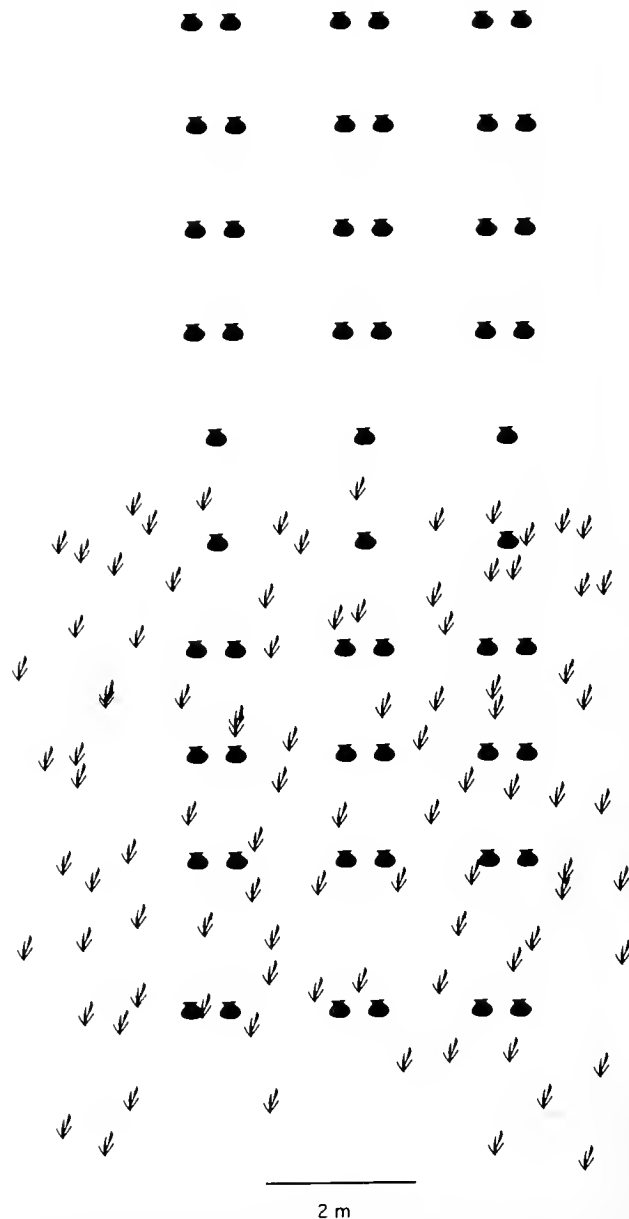


Figure 2. Design of predation experiment. Twenty-seven tagged scallops were placed on bare sand (upper) and 27 were placed in seagrass bed (lower).

within a 24-m radius of the release point at Middleton (Fig. 3), and 52 (58%) scallops were found alive within a 12-m radius of the release point at Deep Bay (Fig. 4). No scallops were found between 24 and 30 m of the release point at Middleton and 12 and 30 m at Deep Bay.

The numbers of scallops in each 30° interval around the release point were grouped to plot circular histograms and for statistical analyses. The circular histogram for Middleton (Fig. 5) indicated a bimodal distribution near the north-south axis. At Deep Bay, the histogram (Fig. 6) indicated a northerly dispersal of *E. bifrons*.

For Middleton scallops, the axis of bimodal dispersal was calculated as $\bar{a} = 011.4^\circ$, with a 95% confidence interval of $\pm 17^\circ$. The hypothesis that the direction of dispersal would be along the axis of current flow was examined using the *V* test. The mean angle predicted was 010° (i.e., that of the current flow). The *V* test indicated that the distribution was significantly different from random ($p < 0.0005$) and that the scallops were clustered along the axis of current flow.

For Deep Bay scallops, the mean angle of dispersal was calculated as north ($\bar{a} = 000^\circ$), with a 95% confidence interval of $\pm 35^\circ$. Rayleigh's test indicated that the dispersal was significantly different from random ($p < 0.005$), supporting the conclusion that there was a mean population direction. The northern direction corresponded with a shoreward dispersal or movement into shallower water.

Predators

The main predators of adult scallops identified in this investigation were the 11-armed spiny starfish *Coscinasterias muricata*, and the octopus *Octopus maorum*. The source of predation of recently killed scallops was identified by the state of shell remains. Starfish generally leave both valves of scallops intact, with the valves held together at the auricle by the hinge ligament and open at the ventral margin (Claereboudt and Himmelman 1996, Hatcher et al. 1996). Octopuses break the lower valve of *E. bifrons* near the

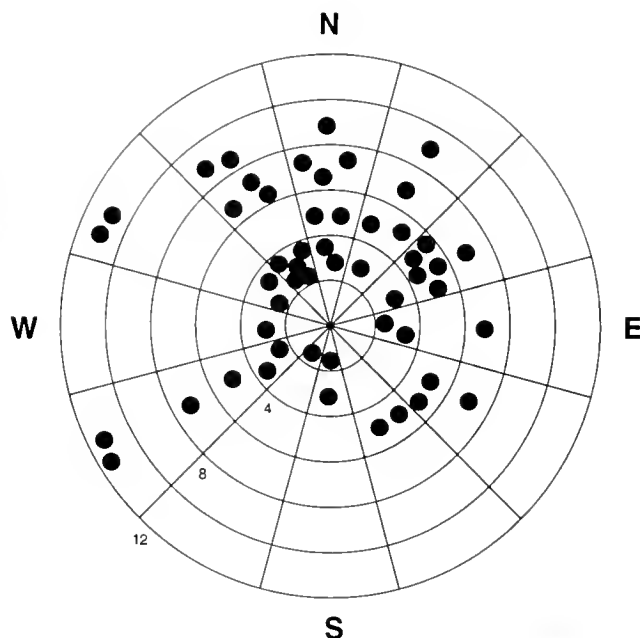


Figure 4. Dispersal of tagged *E. bifrons* from a central release point after about 6 mo at Deep Bay. Concentric circles are 2 m apart.

auricle (pers. obs.). Missing scallops were also assumed to have been taken by octopuses, because of their habit of carrying prey items back to their hole to devour them (Wells 1978). The shells from one tagged scallop were found around an octopus's hole, which supports this assumption. During the tether and tag predation experiments, the source of predation of dead scallops was recorded using these criteria.

Predation on Tethered Scallops

The number of surviving tethered scallops fell rapidly, particularly those placed on sand (Fig. 7). The number of tethered scallops alive in seagrass remained above that for scallops tethered on sand at every sampling date (Table 1). On Days 16 and 28, the number of scallops alive in seagrass was significantly greater than

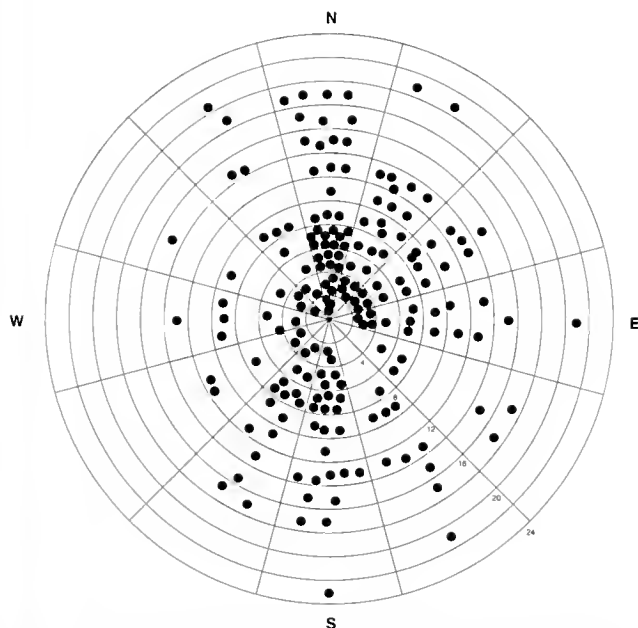


Figure 3. Dispersal of tagged *E. bifrons* from a central release point after 6–7 mo at Middleton. Concentric circles are 2 m apart.

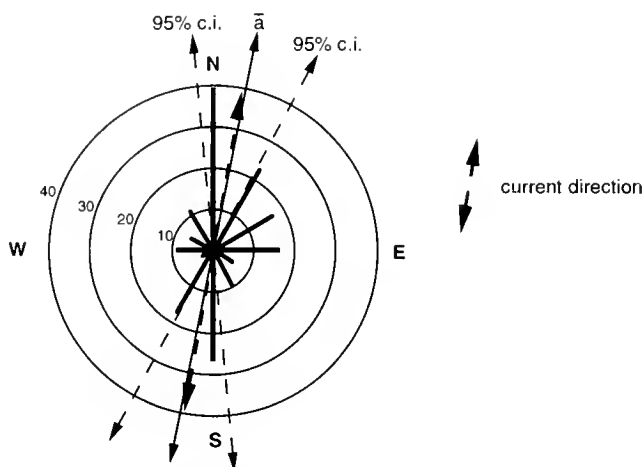


Figure 5. Numbers of *E. bifrons* in each 30° interval around the central release point at Middleton. Mean angle of bimodal dispersal = \bar{a} ; mean angle 95% confidence interval = 95% c.i.

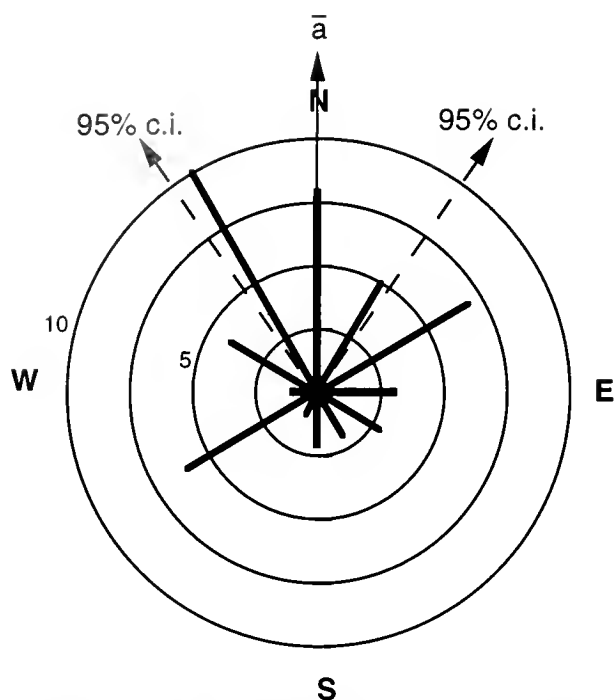


Figure 6. Numbers of *E. bifrons* in each 30° interval around the central release point at Deep Bay. Mean angle of dispersal = \bar{a} ; mean angle 95% confidence interval = 95% c.i.

that on sand ($\chi^2 = 8.33$, $df = 1$, $p < 0.005$; $\chi^2 = 5.00$, $df = 1$, $p < 0.05$, respectively).

Predation on Tagged Scallops

Fourteen tagged scallops (52%) were recovered from the original 27 that were placed on sand. Only seven of these scallops were alive, one of which still remained on sand, while six had moved into seagrass. Twenty-six tagged scallops (96%) were recovered

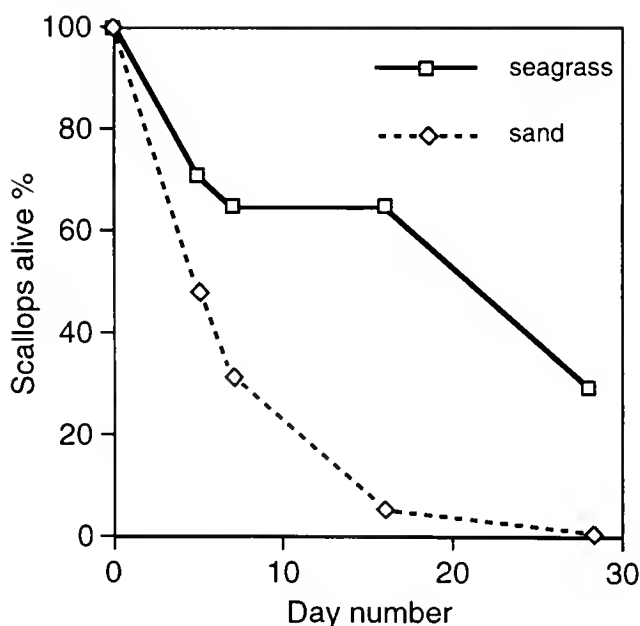


Figure 7. Survival of *E. bifrons* tethered on sand ($n = 19$) and in seagrass ($n = 17$) at Eggs and Bacon Bay.

TABLE 1.

Survival of tethered *E. bifrons* on sand and seagrass at Eggs and Bacon Bay.

Day	Scallops Alive in Seagrass	Scallops Alive on Sand	χ^2	p
0	17	19		
5	12	9	0.43	>0.25
7	11	6	1.47	>0.10
16	11	1	8.33	<0.005
28	5	0	5.00	<0.05

from the original 27 that were placed in seagrass. Only 2 of these scallops were dead, and all 26 were found in seagrass.

Over 49 days, the survival of tagged scallops was significantly greater for scallops originally placed in seagrass than for scallops originally placed on sand ($\chi^2 = 9.32$, $df = 1$, $p < 0.005$) (Table 2). The survival of tagged scallops that were recovered in their original location was also significantly greater for seagrass than for sand ($\chi^2 = 21.16$, $df = 1$, $p < 0.001$).

Source of Predation

Over 28 days, the remains of 3 tethered scallops in seagrass and 10 in sand indicated that the deaths were attributable to starfish predation. The remains of eight tethered scallops in each of the seagrass and sand habitats were attributed to octopus predation; in addition, one tethered scallop was missing from each habitat for a total of nine scallop deaths attributable to octopus predation from each habitat. There were no significant differences between octopus and starfish predation on tethered scallops in seagrass and sand (Table 3).

Over 49 days, the remains of four tagged scallops on sand indicated that the deaths were attributable to starfish predation; no tagged scallops from seagrass were lost to seastar predation. The remains of two tagged scallops from seagrass and three from sand indicated that the deaths were attributable to octopus predation. In addition, 1 scallop from seagrass and 13 from sand were missing for a total of 3 and 16 scallop deaths attributable to octopus predation from each of the respective habitats.

Octopuses killed significantly more tagged scallops from the sand habitat than did starfish ($\chi^2 = 7.20$, $df = 1$, $p < 0.01$) (Table 4). From both habitats combined, octopuses killed significantly more tagged scallops than did starfish ($\chi^2 = 9.78$, $df = 1$, $p < 0.005$).

Tethering Versus Tagging

Scallop mortality rates derived from tethering experiments were markedly higher than those derived from tagging experi-

TABLE 2.

Survival of tagged *E. bifrons* on sand and seagrass at Eggs and Bacon Bay after 49 days.

	Scallops Alive From Seagrass ($n = 27$)	Scallops Alive From Sand ($n = 27$)	χ^2	p
All survivors	24	7	9.32	<0.005
Survivors in location	24	1	21.16	<0.001

TABLE 3.

Predation of tethered *E. bifrons* by starfish and octopuses over 28 days at Eggs and Bacon Bay.

	Scallops Eaten by Starfish	Scallops Eaten by Octopus	χ^2	p
Seagrass (n = 17)	3	9	3.00	>0.05
Sand (n = 19)	10	9	0.05	>0.75
Total	13	18	0.81	>0.50
χ^2	3.77	0.00		
p	0.05			

ments. Scallops tethered in seagrass and sand were killed at a loss rate of 2.5% and 3.6% day⁻¹, respectively, for a combined loss rate across both habitats of 3.0% day⁻¹. Tagged scallops in seagrass and sand were killed at a loss rate of 0.2% and 1.5% day⁻¹, respectively, for a combined loss rate across both habitats of 0.9% day⁻¹. Across both habitats combined, the loss rate in tethered scallops was over three times greater than that for tagged scallops.

DISCUSSION

Movement in Determining Distribution

There have been persistent reports from fishermen around the world of beds of scallops migrating, or at least moving out of an area (Posgay 1981), but investigations of such claims provide little evidence that scallops participate in active, directed migrations. Analyses of tag returns for *Amusium balloti* (Heald and Caputi 1981) and *Amusium japonicum balloti* (Dredge 1985) have suggested a lack of appreciable movement. Howell and Fraser (1984) observed that large numbers of *Pecten maximus* were displaced by only about 30 m after 18 mo. Active swimming species such as *Placopecten magellanicus*, may move greater distances. Movements of more than 60 m were recorded for 49% of seeded *P. magellanicus* after only 44 days (Cliche et al. 1994). There is, however, no evidence of extensive migrations in *P. magellanicus* (Dickie 1955, Posgay 1981, Krantz et al. 1984, Hatcher et al. 1996, Stokesbury and Himmelman 1996). Similarly, results from this work indicate that *E. bifrons* does not move appreciably; over a 6-mo period, few scallops had moved more than 20 m. Movements in this study were probably exaggerated because of the tendency for scallops transplanted at high densities to stimulate one another to swim (Howell and Fraser 1984). Approximately 51 and 42% of tagged scallops were not recovered from Middleton and Deep Bay, respectively. We assumed that these scallops did not move beyond the 30-m radii searched because no scallops were found between the radii 24–30 m at Middleton and 12–30 m at Deep Bay. We attributed the unrecovered scallops to a combination of tag loss and predation.

Directional movements of scallops reported in the literature are generally associated with the axis of strongest current flow. Moore and Marshall (1967) demonstrated that the only directionality in net *Argopecten irradians* movements over short time periods was the consequence of passive transport by tidal currents. Similarly, the direction of dispersion of adult *P. magellanicus* corresponded with bottom-water residual currents (Posgay 1981, Thouzeau et al. 1991). However, more recent work on *P. magellanicus* (Carsen et al. 1996, Hatcher et al. 1996) has shown that the mean direction of displacement does not always correspond with the mean current

direction. In this study, the direction of dispersal of *E. bifrons* at Middleton was along the axis of the strong tidal currents.

At Deep Bay, a random dispersal of scallops was expected because of the negligible current flow at this site. However, *E. bifrons* dispersed in a northern or shoreward direction into shallower water, although the depth difference between the release point and 10 m north of the release point was only about 0.3 m. Up-slope movements have been observed in *P. maximus* (Howell and Fraser 1984, Minchin 1989) and may serve to increase population densities in shallow water to enhance synchronized spawning or be related to better feeding conditions in shallower water (Minchin 1989). In sheltered bays, concentrations of *P. maximus* occur near the tops of slopes, particularly where soft sediments meet rock (Baird 1966, Minchin 1989). A similar distribution occurs for *E. bifrons* in sheltered bays, with concentrations often found along the shallowest edge of seagrass in close proximity to rocks or the sand line (Olsen 1955, pers. obs.).

Predation and Habitat Use

In shallow bays, *E. bifrons* was almost completely restricted to seagrass beds and was rarely found on adjacent sand areas. Predation on tethered scallops was significantly greater on sand than in seagrass. The same phenomenon was deduced from the survival and movements of tagged scallops that were released in the two habitats. If tagged *E. bifrons* did not move into seagrass from sand inside of 49 days, then they would almost certainly be predated upon.

Similarly, in shallow bays, *A. irradians* usually occurs only in seagrass beds and not on nearby open patches of sand (Winter and Hamilton 1985, Smith et al. 1989, Prescott 1990). Winter and Hamilton (1985) investigated this distribution and observed that *A. irradians* swims more frequently and for greater distances when released on sand than in patches of seagrass; after reaching seagrass, most scallops cease swimming. Smith et al. (1989), however, reported that *A. irradians* deployed over bare or transplanted seagrass areas are never found in nearby natural seagrass beds, even when the natural bed is only a few meters distant. Scallops deployed in transplant areas, and in particularly bare areas, suffer a much higher mortality than do those in natural seagrass beds. Smith et al. (1989) suggest that the distribution of *A. irradians* is controlled principally by differential mortality associated with the settlement site (bare area or seagrass bed), rather than by active habitat selection, as suggested by Winter and Hamilton (1985).

This study indicates that predation is important in restricting *E. bifrons* to seagrass in these shallow bays. However, 23% of tagged scallops released on sand were recovered alive in seagrass. This does not necessarily imply that *E. bifrons* is actively seeking out

TABLE 4.

Predation of tagged *E. bifrons* by starfish and octopuses over 49 days at Eggs and Bacon Bay.

	Scallops Eaten by Starfish	Scallops Eaten by Octopus	χ^2	p
Seagrass (n = 27)	0	3	3.00	>0.05
Sand (n = 27)	4	16	7.20	<0.01
Total	4	19	9.78	<0.005
χ^2	4.00	8.89		
p	<0.05	<0.005		

seagrass, because movement in these scallops may have been a result of the escape response to predation (Moore and Trueman 1971, Thomas and Gruffydd 1971, Peterson et al. 1982), which involves violent clapping of the valves, jumping, and swimming until the predator is dislodged (Thomas and Gruffydd 1971), and subsequent random dispersal into seagrass. The role of active habitat selection in this distribution of *E. bifrons* needs to be further investigated. One other factor that needs to be considered is that patterns of adult distribution may be determined by processes occurring during the larval and juvenile stages (Eckman 1987). Seagrass may provide a more suitable settlement site or a better refuge from predation on juvenile scallops, as has been reported for *A. irradians* (Ambrose and Irlandi 1992).

Seagrass beds offer a partial refuge for *E. bifrons* from predation by the 11-armed starfish *C. muricata*, and the octopus *O. maorum*. Seagrass reduces the starfish's mobility (pers. obs.) and therefore decreases the frequency of contact between starfish and scallops. Similarly, the reduction in mobility of two benthic gastropod predators in seagrass has been linked with reduced predation rates on *A. irradians* (Winter and Hamilton 1985). Predation rates of *O. maorum* on tagged *E. bifrons* were less in seagrass areas than in sand. This may be related to the camouflage that is provided by seagrass to scallops, because octopuses recognize their prey by sight (Wells 1978). Camouflage provided by seagrass was claimed by Winter and Hamilton (1985) to be important in reducing predation on *A. irradians*. Because swimming in scallops is primarily triggered by attacks from predators (Winter and Hamilton 1985), a decrease in the frequency of contact between predators and scallops in seagrass would provide an important mechanism for scallops to be retained in seagrass.

In this study, the increased predation rate on tethered scallops (3.0% day⁻¹) compared with tagged scallops (0.9% day⁻¹) highlighted the importance of *E. bifrons* being able to move, or effect an escape response, to avoid predation. Similar investigations have found that tethering in *A. irradians* (Prescott 1990) and *P. magellanicus* (Hatcher et al. 1996, Stokesbury and Himmelman 1996) results in increased predation rates when compared with predation rates in scallops able to swim freely.

The effect of the tethering procedure, however, varies between

predators (Barbeau and Scheibling 1994). The probability of sea stars capturing encountered *P. magellanicus* determines the predation rate. Tethering limits the escape response in *P. magellanicus*, resulting in an increase in the probability of capture and, therefore, predation rate (Barbeau and Scheibling 1994). The same phenomenon was evident in this study by the high predation rate of starfish on tethered *E. bifrons* (1.3% day⁻¹) compared with tagged *E. bifrons* (0.2% day⁻¹). Barbeau and Scheibling (1994) found that in crab and *P. magellanicus* interactions, encounter rate was a major determinant of predation rate and because tethering did not affect encounter rates, it did not affect predation rate by crabs. We believe a similar interaction occurs for octopus and *E. bifrons* encounters. The octopus is a highly mobile predator that can hunt visually, so the escape response would probably be less effective on such a predator; therefore, encounter rate may be important in determining predation rate. The predation rate of octopuses on tethered *E. bifrons* (1.8% day⁻¹) was still more than twice that of tagged scallops (0.7% day⁻¹), indicating that other artefacts of tethering may be operating to increase the encounter rate between octopuses and scallops. We suggest that the tethering procedure, which involved attaching the scallops to a 5-m length of white rope, increased the visibility of scallops to octopuses, which were soon able to learn (Wells 1978) that the rope indicated easy prey. This was reflected in the evenness of octopus predation on tethered scallops in seagrass and sand.

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MITOCHONDRIAL GENOTYPE VARIATION IN A SIBERIAN POPULATION OF THE JAPANESE SCALLOP, *PATINOPECTEN YESSOENSIS* (JAY)

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ABSTRACT Restriction fragment-length polymorphism analysis was used to evaluate genetic variation in a Siberian population of the Japanese scallop (*Patinopecten yessoensis*), and the results were compared with those of a similar study of populations in Japan and British Columbia. The polymerase chain reaction was used to amplify three coding regions of the mitochondrial genome (1.5 kilobases [kb] of the ATP synthetase subunit 6 and cytochrome *c* oxidase subunit 3, a 1.1-kb region including the tRNA gene for threonine, and 1.4 kb of the cytochrome *b* apoenzyme) in 20 scallops collected in Peter the Great Bay (Primorye region of Siberia, Russia). Digestion of each region with 11 restriction enzymes revealed 22 polymorphic sites. Haplotype diversity and within-population nucleotide diversity were high in the Siberian sample (0.98 and 0.015, respectively). Both estimates are much greater than those reported by others for a Canadian hatchery population (haplotype diversity = 0.53, nucleotide diversity = 0.0012) and two Japanese populations (mean haplotype diversity = 0.72, mean nucleotide diversity = 0.0017). Genetic divergence between regions (Japan and Siberia) was calculated on the basis of a subset of restriction sites common to both studies. Estimates of divergence were low (0.004–0.018), and the variation between regions was not significant (analysis of molecular variance, 1.7%; $p = 0.14$). Haplotype frequency distributions, however, were significantly different among regions ($p = 0.028$, log-likelihood exact test).

KEY WORDS: mtDNA variation, Japanese scallop, *Patinopecten*, geographic variation

INTRODUCTION

The Japanese scallop, *Patinopecten yessoensis* (Jay), inhabits coastal areas in the cold waters of the northwestern Pacific Ocean, the southern Sea of Okhotsk, and the Sea of Japan (Fig. 1). It is a commercially important species in both Japan and the Primorye region of Siberia, Russia. Culture of *P. yessoensis* in Japan began in the mid-1960s in response to declining natural stocks. By 1993, the average annual harvest of cultured scallops was estimated as 200,000 tons. *P. yessoensis* culture in Russia has developed at a much slower pace, but between 1984 and 1992, estimated annual harvests increased from 40 to 150 tons (Anonymous 1994).

The commercial importance of the Japanese scallop has led to interest in its stock structure throughout its geographic range. Previous genetic studies have yielded contrasting pictures of regional variation. In an allozyme analysis of six highly polymorphic enzymes (heterozygosities of 0.15–0.53), Pudovkin and Dolganov (1991) found very slight heterogeneity between samples from Sakhalin (Kurile Islands) and the coast of the Primorye region in the Sea of Japan. However, samples within the Primorye region were found to be genetically homogeneous over a distance of 1,200 km. In contrast, Yamanaka and Fujio (1984) found relatively large differences in allozymes between samples collected from the island of Hokkaido and the prefectures of Aomori, Iwate, Miyagi, and Fukushima on the island of Honshu (as reported in Kijima et al. 1984). Their analysis of 22 loci yielded an average genetic distance (Nei's *D*; Nei 1972) estimate of 0.036 among the six localities and 0.068 between the Hokkaido and Honshu samples. Kijima et al. (1984) reported genetic differences in allozyme frequencies among natural (not cultured) populations from the northern Hokkaido coast in the Okhotsk Sea, although the associated distance values (ranging from 0.0006 to 0.0095) were less than 0.01; the level of divergence was associated with differentiated local races (Nei 1972).

More recently, Boulding et al. (1993) used restriction fragment-length polymorphism (RFLP) analysis of mitochondrial DNA cod-

ing regions to compare the genetic diversity of a hatchery population of *P. yessoensis* cultured for three generations in British Columbia with that of its source population from Mutsu Bay, (Aomori), Honshu, and a second wild population from Uchiura Bay, Hokkaido. Their analysis revealed no significant reduction in genetic diversity attributable to low effective population size in the hatchery population, nor was there any evidence of significant divergence among the three sampled populations. It was suggested that gene flow in the form of larval transport or transplantation of newly settled spat was likely sufficient to maintain genetic homogeneity among the Japanese populations.

Although these previous studies have evaluated population differentiation within the two major regions (the Japanese and Siberian coasts) of *P. yessoensis* abundance, similar comparisons between the two regions are lacking. The objective of our study was to use the data produced by Boulding et al. (1993) on mitochondrial DNA diversity in Japanese populations of *P. yessoensis* for comparison with similar data collected on a sample from Peter the Great Bay, Siberia, Russia.

METHODS

Collection Sites

Siberian scallops were collected by SCUBA from 15 to 20 m in Amursky Bay, which is part of Peter the Great Bay (43°N, 132°E) in the northern Sea of Japan (Fig. 1). Sampled adductor muscle was frozen and shipped within a few days of collection in the spring of 1993. After receipt, samples were stored at –80°C until DNA extraction. E. Boulding provided mtDNA extracts for nine individuals from the previously examined Japanese populations to facilitate standardization of haplotype designations between studies. Five of the extracts were derived from scallops collected in Uchiura Bay, (Hokkaido) Japan (42°N, 141°E), and the remaining four came from Mutsu Bay, Aomori (Honshu), Japan (40°50'N, 140°46'E).

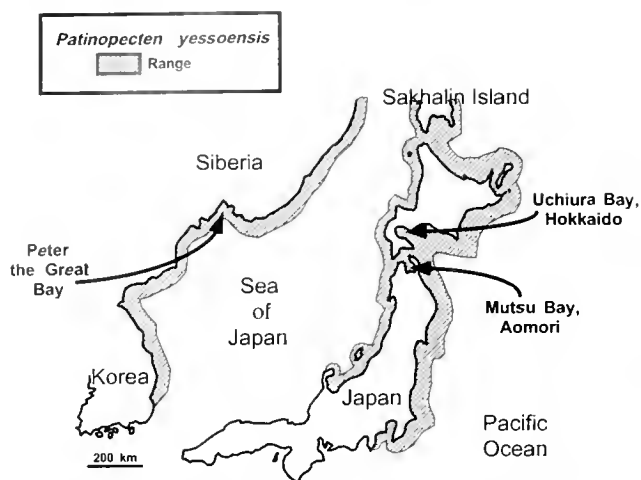


Figure 1. Range of *P. yessoensis* in the northwest Pacific ocean. Origins of samples indicated by arrows.

DNA Extraction and Amplification

DNA was extracted from adductor muscle from the Siberian scallops by a guanidine thiocyanate method (Puregene, Gentra Co.) following manufacturer's specifications. Primers were synthesized according to sequences reported in Boulding et al. (1993) and were specific for three coding regions of the mitochondrial genome: (1) EB48/EB49 amplified a ≈ 1.5 -kilobase (kb) portion of the ATP synthetase subunit 6 and cytochrome *c* oxidase subunit 3 (hereafter, COIII), (2) EB59/EB60 amplified a ≈ 1.4 -kb fragment of the cytochrome *b* apoenzyme (hereafter, Cytb), and (3) EB53/EB54 amplified a ≈ 1.1 -kb fragment containing the transfer RNA for threonine and an unidentified reading frame (hereafter, tRNA). The three fragments were amplified from 20 Siberian, 5 Hokkaido, and 4 Aomori scallop extracts. Amplifications were performed on a Perkin-Elmer 480 thermocycler, and the 100- μ L reactions contained 1.6 mM $MgCl_2$, 200 μ M deoxynucleotide triphosphates, 0.3 μ M specific primers, and 2.5 U of *Taq* polymerase. Cycling conditions consisted of 30 cycles of 30 sec at 94°C, 30 sec at 48°C, and 90 sec at 72°C.

Restriction Digest and Electrophoresis

Digestion of the polymerase chain reaction (PCR) products with restriction endonucleases was performed according to the manufacturer's specifications (New England BioLabs), with 10 μ L of product exposed to 5 U of enzyme in a 20- μ L total volume. Digests were incubated for 3 h and stopped with 5 μ L of loading dye (20% Ficoll 400, 0.1 M Na EDTA [pH 8], 1% sodium dodecyl sulfate, 0.25% bromophenol blue). Entire digests were loaded onto 19-cm 2% agarose gels and electrophoresed for 5 h at 65–70 V. Fragment patterns were visualized by ethidium bromide staining and photographed under ultraviolet light. Fragment sizes were determined from migration distances relative to known standards. All three PCR products were digested with a battery of 11 enzymes: *AhaI*, *DdeI*, *DraI*, *HaeIII*, *HhaI*, *HinI*, *MspI*, *RsaI*, *Sau3AI*, *Sau96I*, and α *TaqI*.

Statistical Analysis

Haplotype data generated in this study were analyzed with the Restriction Enzyme Analysis Package (REAP version 4.0) (McEl-

roy et al. 1992). Sample haplotype diversities were calculated following Nei (1987). Nucleotide diversities were computed according to Nei and Miller (1990). A haplotype network depicting the relationships among haplotypes observed in this study was constructed using the minimum spanning tree algorithm provided in NTSYS-pc (version 1.7, Rohlf 1992).

Haplotype information on an additional 12 Hokkaido, 6 Aomori, and 25 Nanaimo hatchery scallops, as reported in Boulding et al. 1993, was used for comparisons among populations (data courtesy of E. Boulding). These data consisted of haplotype designations for the enzyme/PCR product combinations that were polymorphic in an initial screening of 16 individuals: *HhaI*/COIII, *MspI*/COIII, *Sau96I*/COIII, α *TaqI*/COIII, *HaeIII*/Cytb, *MspI*/tRNA, and *Sau96I*/tRNA. Because most of the individuals from the Japanese populations were not examined for all enzyme/PCR product combinations, our comparative analysis was constrained to the subset of sites that was scored in all individuals from all populations. This data subset consisted of 20 sites, 9 of which were polymorphic.

Analysis of the expanded population samples (including data provided by Boulding) for this subset of polymorphic sites included estimation of divergence following Nei (1987). Haplotype frequency distributions were analyzed with a log-likelihood exact test (StatExact). Populations were then nested into three geographic regions (Siberia, Japan, and British Columbia) and subjected to an analysis of molecular variation (AMOVA) (Excoffier et al. 1992). AMOVA allows estimation of the fraction of genetic variance attributed to different hierarchical levels based on the geographic distribution of haplotypes and a matrix of squared distances between all pairs of haplotypes. AMOVA also uses the distance estimates between haplotypes to calculate haplotypic correlations at different hierarchical levels (among geographical regions, among populations within regions, and within populations). These correlations (ϕ statistics) quantify the degree of population structure at each level, in a manner analogous to the hierarchical *F*-statistic analysis of Cockerham (1969, 1973).

RESULTS

Fragment sizes resulting from the digestion of the three PCR-amplified mtDNA regions are shown in Table 1. Underlined estimates were not visualized because of the lack of sufficient resolution in ethidium-stained agarose gels but were inferred because of the discrepancy between the size of the undigested PCR product and the sum of the visualized fragments. A total of 94 sites was observed, 22 of which were polymorphic.

Haplotype frequencies resulting from digestion of all three mtDNA fragments are reported in Table 2. Haplotype diversity and within-population nucleotide diversity for the Siberian population were relatively high, 0.9942 and 0.0060, respectively. The nine samples from Hokkaido and Aomori analyzed in our study were selected with the intent of illustrating the breadth of variation uncovered by Boulding et al. (1993) and therefore do not represent a random sample of population variation. Consequently, similar haplotype statistics were not calculated for the Japanese populations based on our analysis. However, the haplotype and nucleotide diversity estimates reported by Boulding et al. (1993) for these Japanese populations were much less (haplotype diversity: Hokkaido [$n = 14$] = 0.79, Aomori [$n = 10$] = 0.66; nucleotide diversity: Hokkaido = 0.0021, Aomori = 0.0012) (Boulding et al. 1993). It should be noted that such values are sensitive to sample

TABLE 1.
Fragment patterns produced by digestion of PCR products with restriction endonucleases.

PCR	Product: COIII	Cytochrome <i>b</i>	tRNA
<i>AluI</i>	A: 460 + 250 + 200 + 90	A: 1040 + 250 + 110 B: 900 + 140 + 250 + 110 A: 410 + 350 + 430 + 120 + 90	A: 570 + 350 + 180 A: 480 + 370 + 250 B: 480 + 240 + 130 + 250 A: 1100 B: 900 + 200 A: 600 + 260 + 240
<i>DdeI</i>	A: 660 + 490 + 150 + 130 + <u>70</u>	B: 760 + 430 + 120 + 90	
<i>DraI</i>	A: 750 + 400 + 350	A: 890 + 510	
<i>HaeIII</i>	A: 370 + 280 + 270 + 260 + 210 + 110 B: 370 + 280 + 270 + 140 + 120 + 210 + 110	A: 940 + 270 + 190 B: 640 + 300 + 270 + 190	
<i>HhaI</i>	A: 540 + 530 + 430 B: 540 + 440 + <u>90</u> + 430	A: 570 + 560 + 270	A: 1100 B: 970 + 130
<i>HintI</i>	A: 560 + 560 + 380	A: 530 + 330 + 30 + 200 + 160 + 150 B: 530 + 410 + 200 + 160 + 150	A: 502 + 299 + 168 + 148
<i>MspI</i>	A: 1500 B: 1220 + 360	A: 670 + 280 + 210 + 190 + <u>50</u>	A: 700 + 280 + 120 A: 630 + 470 B: 420 + 210 + 470 C: 630 + 200 + 270
<i>Sau3AI</i>	A: 910 + 450 + 140	A: 1400 B: 740 + 660 A: 1400 B: 680 + 290 + 230 + 200 C: 410 + 270 + 290 + 230 + 200 D: 680 + 290 + 430	
<i>RsaI</i>	A: 530 + 450 + 300 + 220 A: 820 + 340 + 340 B: 1160 + 340	A: 560 + 380 + 140 + 130 + 110 + 80 B: 280 + 280 + 380 + 140 + 130 + 110 + 80	A: 940 + 160 B: 720 + 220 + 160 A: 650 + 260 + 190 B: 380 + 270 + 260 + 190
<i>Sau96I</i>	C: 680 + 140 + 340 + 340	A: 520 + 290 + 240 + 190 + 160	A: 1100
α <i>TaqI</i>	A: 540 + 460 + 350 + 150		

Fragment sizes estimated by migration relative to known standards. Underlined fragments were inferred from discrepancies between the uncut PCR product and the sum of the visualized pieces.

sizes, and thus, values based on different numbers of individuals should be compared with caution. Further, the analysis of the Japanese populations was based on fewer polymorphic sites than that of the Siberian population.

Construction of a minimum spanning tree based on calculated genetic distance between haplotypes observed in this study revealed that the majority of haplotypes were closely related and that adjacent haplotypes generally differed from one another by only one or two site changes (Fig. 2). The haplotypes do not show strong geographic structuring, in that haplotypes unique to particular populations are not necessarily more closely related to other haplotypes from the same population.

The subset of restriction sites used in the analysis of population divergence produced 11 distinct haplotypes in the 17 Hokkaido, 10 Aomori, 25 Nanaimo, and 20 Siberian scallops analyzed (Table 3). Divergence values among populations were small, with the Hokkaido population being as divergent from the geographically adjacent Aomori populations as it was from the much more distant Siberian population (Table 4). The Nanaimo hatchery population was the least distant from its source population in Aomori, but generated the largest divergence value observed in this study when compared with the Siberian population.

Analysis of the haplotype frequency distributions of the Japanese populations and the Nanaimo hatchery population for this subset of restriction sites yielded the same conclusion reported in Boulding et al. (1993): there are no significant differences among these populations ($p = 0.219$, log-likelihood exact test). However, the comparison between the Siberian and the pooled Japanese (Aomori and Hokkaido) haplotype distributions was significant

($p = 0.028$), suggesting that these two regions do not possess identical mitochondrial gene pools.

The results of the AMOVA of the four populations nested by geographic region indicated that the overwhelming majority of the variance was due to within-population variation (Table 5). Less than 2% could be attributed to variation among geographic regions, which was not found to be significant (AMOVA, $p > 0.1$).

DISCUSSION

The results of the statistical analysis of mtDNA variation provide contrasting pictures of stock structure in *P. yessoensis* in the northwestern Pacific Ocean. The lack of significant divergence and the close relationships of the various haplotypes suggest that there has been significant and recent gene flow among these populations. Estimated rates of migration (N_m) based on Φ_{ST} values range between 14 and 71 individuals per generation between Japan and Siberia and reflect rates in excess of the theoretical minimum value of four that is necessary to prevent differentiation by drift under either an island or one-dimensional stepping stone model of population structure (Kimura and Maruyama 1971, Nagylaki 1983).

In contrast, the significant difference in haplotype distributions suggests that the regions do not possess identical gene pools and that actual levels of gene flow may be somewhat more restricted between Siberia and Japan than is suggested by the estimates of N_m presented above. These regional differences are apparent despite the fact that the comparative analysis involves a subset of the observed mitochondrial variation in the Siberian sample, and relatively small sample sizes overall. The frequency distributions

TABLE 2.

P. yessoensis: haplotype frequencies for samples from Japan and Siberia.

Code	Composite Haplotype	Frequencies	
		Japan	Siberia
J1	AAAAABAAABAABAAAA	1	0
J2	AAABAAABABAABAAAB	1	0
J3	ABAAAAABAABAABAAAA	1	0
J4	AAAAAAAAABAABAAAA	1	0
J5	AABAAABBBBAABAAAA	1	0
J6	BAACAABAABAABAAAA	1	0
J7,S1	AAAAABAABAABAAAA	1	3
J8	AAAAABAABAABACAA	1	0
S2	AAAAABAABAABAAAA	1	0
S3	AAAAABBABBAABAA	0	1
S4	AAAAAABABBABAAAA	0	1
S5	AAAAAABBBBAABAAAA	0	1
S6	AAAAAABABBAABAAAA	0	1
S7	AABAAABAABAABAAAA	0	1
S8	AAAAAABABAABAAAA	0	1
S9	AAAAAABAAAAABAAAA	0	1
S10	AAAAAABBAABABAAAA	0	1
S11	AABAAAAAABAABAAAA	0	1
S12	AAAAAABBBBAABAAAA	0	1
S13	AAAAAABBABAAAAAA	0	1
S14	AAAAAABAADAAAAAA	0	1
S15	AAAAAACAABAABAAAA	0	1
S16	AAAAAABAABAAAAAA	0	1
S17	AABAAAAAABAABAAAA	0	1
S18	AAAAAABAABABAAAA	0	1
S19	AAAAAABAABABAAAA	0	1

Letter designations correspond to fragment patterns described in Table 1. For simplicity, only patterns for the polymorphic enzymes are indicated in the composite haplotypes. Codes identify haplotypes for use in Figure 2. Composite designations are as follows: COIII: *Hae*III, *Hha*I, *Msp*I, *Sau*96I; CYTb: *Alu*I, *Dde*I, *Hae*III, *Hinf*I, *Sau*3A1, *Rsa*I, *Sau*96I; tRNA: *Dde*I, *Dra*I, *Hha*I, *Sau*3A1, *Rsa*I, *Sau*96I.

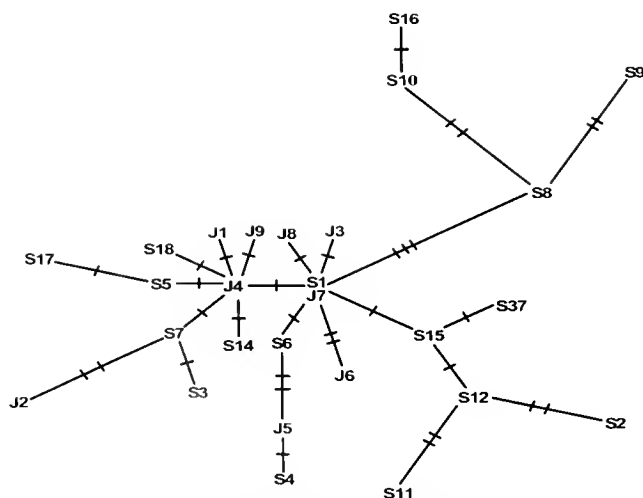


Figure 2. Haplotype network depicting the relationships of the 26 haplotypes generated in this study. Branch lengths were generated by the minimum spanning tree algorithm provided in NT-SYS (Rohlf 1992), based on genetic distances. Haplotype codes are defined in Table 2. Haplotype letters indicate geographic origin. J = Japan, S = Siberia. Hatch marks indicate the number of site changes between adjacent haplotypes.

TABLE 3.

Haplotype frequency distributions for the subset of 20 restriction sites used in the regional comparison of Siberian and Japanese *Patinopecten* populations, including data from Boulding et al. (1993) (see Methods).

Composite Haplotype	Haplotype Frequencies in:			
	Hokkaido	Aomori	Nanaimo	Siberia
AAAAAAA	2	0	0	6
BABAAAA	2	0	0	1
BAAAAAA	6	7	14	11
AAAAAAB	4	1	5	0
BAABAAA	1	1	4	0
AABAAAA	0	0	0	2
AAAABBA	1	0	0	0
BBAAAAA	1	0	0	0
AAAAACA	0	1	0	0
BAAAAACA	0	0	1	0
BAAAAAB	0	0	1	0

Composite designations indicate RFLP for the following enzyme/PCR product combinations: *Hha*I/COIII, *Msp*I/COIII, *Sau*96I/COIII, *α*TaqI/COIII, *Hae*III/Cytb, *Msp*I/tRNA, and *Sau*96I/tRNA.

shown in Table 2 indicate the potential presence of additional regional differences had all 94 polymorphic sites been analyzed in all individuals. Such an extended analysis would likely provide a clearer picture of stock structure in *P. yessoensis*.

Further evidence of stock differentiation is illustrated in Table 2 and Figure 2, which show that the majority of haplotypes observed in the Siberian population are unique. This high frequency of unique haplotypes suggests that current levels of gene flow between the regions are low, and that the lack of divergence revealed by the comparative analysis could reflect historic exchanges between Japanese and Siberian populations of scallops.

The potential for gene flow between the populations in Siberia and northern Japan exists in the form of dispersing planktonic larvae, which persist in the water column for approximately 1 mo before settlement (Ito 1991). These populations, however, are geographically separated by at least 750 km of open ocean, and the prevailing current patterns in the Sea of Japan could serve as a major obstacle to larval exchange (Fig. 3). The northwest flow of the Tsushima current across the Sea of Japan and through the Tsugaru Strait between the islands of Hokkaido and Honshu could prevent the dispersal of *P. yessoensis* larvae from the Japanese to the Siberian populations. The warmer temperature (~24°C in summer) and higher salinity (33–36‰) of the Tsushima current relative to the surrounding waters exceed the conditions for the opti-

TABLE 4.

Estimated divergence values (Nei 1987, eqs. 10–21) between Japanese and Siberian populations of *P. yessoensis* based on the subset of 20 restriction sites (see Methods).

	Hokkaido	Aomori	Nanaimo
Aomori	0.000041		
Nanaimo	0.000090	–0.000352	
Siberia	0.000039	0.000187	0.000878

Data on Hokkaido, Aomori, and Nanaimo hatchery populations from Boulding et al. (1993). Sample sizes are as follows: Hokkaido, n = 17; Aomori, n = 10; Nanaimo hatchery, n = 25; Siberia, n = 20.

TABLE 5.

Results of the AMOVA on the subset of 20 restriction sites using data on Hokkaido, Aomori, and Nanaïmo hatchery populations from Boulding et al. (1993) and data on the Siberian population from this study.

Variance Component	Variance	% Total	Φ Statistic	p Value*
Among regions	0.0096	1.70	Φ_{cr} :0.017	0.5465
Among samples/ within regions	0.0060	1.07	Φ_{sc} :0.011	0.3966
Within samples	0.5476	97.22	Φ_{st} :0.028	0.1099

* p-Value indicates the probability of more extreme random values based on 1,000 permutation tests.

mal development of *P. yessoensis* larvae and consequently may limit successful transport of larvae from the Siberian coast to Japan (Tomeczak and Godfrey 1994, Ito 1991, Kalashnikov 1991). Coastal currents in Peter the Great Bay and the vertical migrations of *P. yessoensis* larvae relative to tidal flow could retain larvae near their parental populations along the Siberian coast (Maru et al. 1973). These physical characteristics of the region could form effective barriers to larval exchange, resulting in the differentiation of regional stocks.

Although this analysis of mtDNA variation does not provide clear evidence of the existence of regional stocks of *P. yessoensis* in the northwest Pacific, the results do suggest that the alternate hypothesis of a single homogeneous stock is not applicable. The differences in the haplotype distributions, and the relatively high haplotype diversity of the Siberian population, suggest that these regions might be somewhat isolated and that different forces might be influencing the genetic make-up of the respective regions. It may be that the long history of artificial propagation and transplantation has altered the genetic make-up of the Japanese popu-

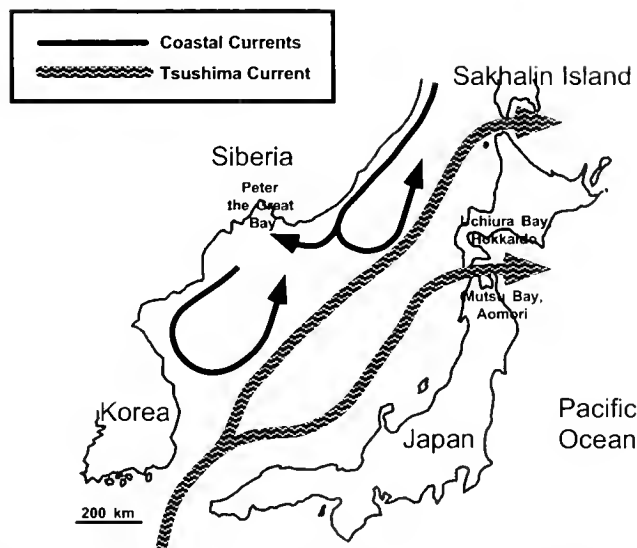


Figure 3. Dominant current movements through the Sea of Japan.

lations. A more extensive analysis of additional populations from both regions would contribute greatly to our understanding of population structure in this species, as well as the possible effects of extensive commercial propagation.

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AMOUNTS OF POLYMORPHISM AT MICROSATELLITE LOCI IN THE SEA SCALLOP *PLACOPECTEN MAGELLANICUS*

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ABSTRACT Seven microsatellite DNA markers have been developed for the commercially important scallop *Placopecten magellanicus*. For four of these loci, the core sequence consists of tandemly repeated dinucleotides (GA). For the other three loci, the core consists of trinucleotide or tetranucleotide repeats with or without intervening sequences. An analysis of a full-sib family produced results compatible with Mendelian transmission and also produced evidence for linkage between two of the loci. A sample from a commercially harvested population produced results that varied markedly among loci. Most of these interlocus differences can be explained in terms of the repeat unit. The four loci with dinucleotide repeats produced comparable results with regard to the observed number of alleles, observed heterozygosities, and range and distribution of allele length. The three loci whose variation has a more complex basis differed from each other and from the other four loci in one or more of these aspects. Excess of homozygosity, however, appears to vary irrespective of core sequence. The large number of alleles and the ability to score larvae and juveniles make this set of markers a powerful instrument for the study of both natural and cultured populations of this species.

KEY WORDS: microsatellites, scallops, *Placopecten magellanicus*

INTRODUCTION

Since the seminal papers of Hubby and Lewontin (1966) and Lewontin and Hubby (1966), molecular genetic markers have become indispensable tools in the study of natural populations as well as in experiments with laboratory or domesticated populations (Avisé 1994). This is even more so with marine organisms, where direct observation of behavior, breeding structure, and migration patterns is more difficult than for most terrestrial organisms (Avisé 1987).

The sea scallop, *Placopecten magellanicus*, forms discrete populations or "beds" along the Atlantic Coast of North America from the northern reaches of Newfoundland to the Virginia Capes. It is one of the most important species for the fishing industries of Atlantic Canada and the eastern United States (Naidu 1991). Because of its high commercial value, it has also been targeted as a potential species for large-scale aquaculture and resource enhance-

ment (Beaumont and Zouros 1991, Naidu 1991). Consequently, much attention has recently focused on the development of genetic markers that could aid in the development of the culture of this species and also help address questions about genetic discontinuity and self-sustenance of the various commercially important scallop beds that presently are being treated as individual "stock units" (e.g., Robert et al. 1993).

Previous population discrimination studies in *P. magellanicus* have used morphometric analysis of the upper shell (Kenchington and Full 1994), allozyme polymorphism (review in Beaumont and Zouros 1991), size polymorphism of mitochondrial DNA (Fuller 1991), and random amplified polymorphic DNA (RAPD) markers (Patwary et al. 1994). A set of anonymous complementary DNA (cDNA) probes have also been developed for this species (Pogson 1994, Pogson and Zouros 1994) but have not as yet been used in population discrimination studies. All of these assays have a number of disadvantages for use as population markers, relating to

technical difficulties, genotype discrimination, or restricted amount of polymorphism (e.g., Hadrys et al. 1992, Lewis and Snow 1992, Riedy et al. 1992).

Microsatellites are free of most of these disadvantages and at the same time have unique properties that make them ideal tools for population genetic studies (for a most recent review for this use of microsatellites, see O'Reilly and Wright 1995). Here, we describe the first microsatellite markers in *P. magellanicus* and provide information on their Mendelian segregation and linkage association. We also present data from a natural population that suggest that levels and patterns of polymorphism vary substantially among loci. We have asked how these differences may relate to the fact that some of our microsatellite loci have a simple dinucleotide repeat core and others have a compound trinucleotide or tetranucleotide core. This preliminary study shows that the microsatellite assays that we have developed would be useful for the study of contained populations and genetic applications related to the aquaculture of this species, as well as for the study of its natural populations.

MATERIALS AND METHODS

Samples

Sea scallop, *P. magellanicus*, samples used in this study were collected by the Department of Fisheries and Oceans, Canada. Twenty-five individuals from Browns Bank, Atlantic Canada (42.8°N 66.3°W), were collected in May 1992, and another 30 were collected in May 1993. These animals were used to obtain an indication of the amount of variation in microsatellite loci occurring in natural populations. Mendelian inheritance and linkage associations were tested on 12 randomly selected offspring from the same pair-mating used by Patwary et al. (1994) for the study of RAPD markers.

DNA Extraction

DNA was extracted essentially as described by Patwary et al. (1994), with a few modifications. Approximately 0.1 g of adductor muscle tissue was ground to a fine powder in liquid nitrogen and mixed with 750 μ L of lysis buffer (10 mM Tris-HCl [pH 8.2], 1 mM Na₂EDTA, 400 mM NaCl). Solutions of sodium dodecyl sulfate (SDS) and Proteinase K (ICN Biomedicals) were added to final concentrations of 0.75% and 100 μ g mL⁻¹, respectively. Samples were mixed and digested at 55°C overnight. Proteins present in the lysate were precipitated by the addition of 250 μ L of saturated NaCl, vortexed for 3 min, and pelleted by centrifugation at room temperature at 800 rpm for 30 min. DNA present in the supernatant was extracted with 400 μ L of chloroform, precipitated with an equal volume of isopropanol, and collected by centrifugation at 14,000 rpm for 15 min. The pellet was washed with 70% ethanol, vacuum dried for 2 min, and resuspended in 50–100 μ L of TE buffer (10 mM Tris-HCl [pH 8.2], 0.1 mM Na₂EDTA).

We also used a rapid protocol for DNA extraction that produced templates of sufficient quality for the polymerase chain reaction (PCR) amplification. Approximately 1 mg of adductor muscle tissue was added to 200 μ L of lysis buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 0.5% Tween-20) and 5 μ L of Proteinase K (20 mg/ml), briefly vortexed, and left at 55°C for several hours to overnight. Proteinase K was inactivated by treatment for 5 min at 95°C. After letting the samples cool down to ambient temperature, tubes were spun to pellet any remaining debris. One

to 2 μ L of the supernatant was used directly as a template for PCR. This protocol results in a more rapid extraction of DNA from small amounts of tissue.

Microsatellite Primer Development

A partial genomic library of *P. magellanicus* DNA was constructed by ligating 300–700 base-pair (bp) scallop DNA fragments into puc18/*Sma*I-BAP vector (Pharmacia). These fragments were generated by digesting total genomic DNA extracted from an individual adductor muscle with the enzymes *Hae*II, *Rsa*I, *Hinc*II, and *Alu*I (Pharmacia) and were size selected on 1% low-melting-point agarose gels. MAX Efficiency DH5 α competent cells (Gibco BRL) were transformed according to manufacturer's instructions. Colonies were transferred to Hybond-N nylon membranes (Amersham) according to manufacturer's instructions and hybridized with [γ -³²P]ATP-labeled synthetic oligonucleotide probes. Hybridizations were carried out overnight at 58°C [for (GA)₁₅ probe] to 65°C [for (GACA)₆ and/or (CAC)₁₀ probes], in 5 \times SSPE, 5 \times Denhardt solution (0.1% w/v Ficoll, 0.1% w/v polyvinylpyrrolidone, 0.1% w/v bovine serum albumin), 0.5% SDS, and yeast tRNA to a final concentration of 100 μ g/mL of hybridization solution. After hybridization, membranes were washed at ambient temperature, twice for 10–15 min in 2 \times SSPE–0.1% SDS and once for 10–15 min in 1 \times SSPE–0.1% SDS. Positive colonies were selected and submitted to secondary screening with the same oligonucleotide probe or mixture of probes.

Approximately 55,000 clones of a partial library were screened, and 215 of these were sequenced by the dideoxy chain-termination method with the T7-sequencing kit from Pharmacia. Seven sequences containing microsatellite motifs were chosen, and primer sequences were designed with the software package Gene Runner version 3.0 (Hastings Software Inc.).

Detection of Microsatellite Variation

Radioactive PCR amplification of scallop microsatellites was carried out in a PTC-100 DNA thermal cycler (MJ Research Inc.). Ten microliters of standard PCR reaction mix contained 10–15 ng of DNA template, 0.6 μ M [γ -³²P]ATP-labeled forward primer, 0.6 μ M reverse primer, 1 mM MgCl₂, 200 μ M dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin, and 0.3 U of *Taq* polymerase. The mixture was overlaid with a drop of mineral oil. Samples were amplified through 25 cycles, each consisting of 20 sec at 94°C, 20 sec at an optimal annealing temperature (see Table 1), and 20 sec at 72°C. The extension step at 72°C was increased to 30 sec per cycle for the primer set Pma-135. Ten microliters of stop dye (10 mM NaOH, 99% formamide, 0.1% bromophenol blue, and 0.1% xylene-cyanol) was added to the amplified DNA. After denaturation for 15 min at 94°C, 3 μ L of mixture was loaded and electrophoresed on an 8% polyacrylamide wedge sequencing gel. The M13mp18 (+) sequence was used as a size ladder.

Statistical Analysis

The two samples from Brown's Bank were tested for homogeneity in allele frequency distribution across loci. The probability varied among the seven loci scored from 0.14 to 0.50, and Fisher's combined probability from independent tests was 0.144. On the basis of this result, we have combined the two samples into one.

For each locus, we recorded the number of scored individuals, the number of observed alleles, the relative length of each allele,

TABLE 1.

Core microsatellite sequences, primer sequences, and PCR conditions for seven microsatellite loci in *P. magellanicus*.

Locus	Core Sequence	Primers (5' to 3')	T _A (°C)
Pma-130	(GA) ₂₂	CCGGATTGTATTGAAGTCT CCATCCTGAATCCTCTTACGA	51 (49*)
Pma-200	(GA) ₃₁	TATACGCACTCAATCACCC GTTGCGTGATCCNCCTG	49
Pma-212	(GA) ₁₆	TAAACTGCTTTGTGGGATG GAATTTGACCTAGAG ACCAG	49
Pma-275	(GA) ₂₃	GAGAAAGTTAGTGTGTGAATG AGAAATGCTTCTCTGCACC	51
Pma-135	(GACA) ₁₀ [(N) ₆ (GACA)] ₃	GTGCACAAATTACAACAAGTCAA ATGATTTGACGATACTACCAGATG	57
Pma-180	(N) ₄₅ GACA (GACA) ₆ (N) ₂₀ (GACA) ₃	ACAAAGTCACATACCGACG TTGTAGTTATTAGTCTATAGGT	50
Pma-132	(GTT) ₉ (GGT) ₃	GACGGTTTTGTTTACATCTCGG CCTATATTCATCATCCATTTAATCCA	50

* T_A (optimal annealing temperature) for scallop larvae with this primer set = 49°C.

and the number of observed heterozygotes. Unbiased estimates of heterozygosity were obtained from:

$$h_{ij} = 2N_j (1 - \sum p_{ij}^2) / (2N_j - 1)$$

where N_j is the number of individuals scored for locus j , and p_i is the frequency of the i th allele at the j th locus (Nei 1987).

The exact binomial probability was used to test for Mendelian segregation. Fisher's exact test for 2×2 tables was used to test for independent allele segregation for pairwise combinations of loci. Conformity of genotype frequencies to Hardy-Weinberg equilibrium was estimated using the GENEPOP software package version 1.2 (Raymond and Rousset 1995).

RESULTS

Detection of Microsatellite Variation

Many of the microsatellite repeats that we isolated from the scallop genome were not suitable as markers. In several of these, the beginning of the repeat array happened to be very close to the cloning site; in others, the array of repeats was too long, with an expected PCR product larger than 300 bp. In others, the flanking regions had poly-A or poly-T sequences, or the repeatability of amplification was not satisfactory. Table 1 gives information about seven microsatellite markers that meet the criteria for genetic markers. Of special importance is that the seven loci form three classes with regard to the core sequence. The first class contains four loci (Pma 130, 200, 212, and 275), the core of which consists of perfect dinucleotide repeats (GA). The second class contains two loci, the core of which consists of interrupted repeats of the tetranucleotide GACA (Pma 135 and 180). The third class is represented by one locus (Pma 132), the core of which consists of two kinds of trinucleotide repeats.

The three classes of loci produce distinctly different patterns of allelic products after electrophoresis on polyacrylamide gels. In the first class, the main band of an allele is followed by the usual "band stuttering" and there is some, but generally small, difference in the intensity of the two major allelic bands. In the second and third classes, there is little or no band stuttering, but there is a noticeable difference in the intensity of allelic bands, with the

shorter allele producing a much stronger band. These differences between the two classes of loci are particularly noticeable for locus Pma-135, where there are two discontinuous classes of allelic lengths. Indeed, accurate determination of length of the large size class alleles was not always possible for this locus. Because of their size discontinuity, the two allelic classes of Pma-135 can be misinterpreted as belonging to two different loci that are amplified by the same set of primers. This interpretation was rejected from the segregation analysis of a pair mating (see below).

Segregation and Linkage Analysis

The evidence that microsatellite alleles in general follow Mendelian segregation is overwhelming, so that most studies reporting new microsatellite assays do not test for Mendelian segregation. We took advantage of the availability of DNA extracts from parents and 12 offspring from a pair-mating that was previously used to test for segregation of RAPD polymorphisms (Patwary et al. 1994) to examine if our microsatellites conform to Mendelian expectation and also to see if there is evidence, in this limited set of family data, for linkage association between any two loci.

The genotypes of the two parents and 12 randomly chosen progeny from a full-sib family are given in Table 2 for all loci except Pma-200, which was not scored satisfactorily in this family. The ratio of parental alleles among offspring is compatible with Mendelian segregation in all loci except in Pma-275, where the two paternal alleles are found in the ratio 8:1 (which is different from the 1:1 ratio at $p = 0.02$). Given that we have segregation observations from nine parent-locus combinations (both parents for Pma-130, Pma-135, and Pma-212; only the female parent for Pma-132 and Pma-180; and only the male parent for Pma-275), the Bonferroni correction (Sokal and Rohlf 1995) for seeing one significant value at the $\alpha = 0.05$ level is 0.006, which is lower than the observed significance level. Thus, on the basis of this set of data, we cannot establish whether Mendelian segregation is violated at locus Pma-275 or the observed deviation is accidental.

The small number of progeny scored allows for the detection of linkage only if the recombination distance between two loci is small. Because each parent was heterozygous at five of the seven loci, the pair-wise combinations of nonallelic genes in female or

TABLE 2.
Microsatellite genotypes in a full-sib family of *P. magellanicus*.

Group	Pma-130	Pma-132	Pma-135	Pma-180	Pma-200	Pma-212	Pma-275
Female	136/150	226/232	276/B†	119/120	-/-‡	98/130	104/104
Male	146/150	200/200	272/A	119/119	-/+	118/122	92/104
Progeny*							
1	150/146	232/200	B/272	119/119	-/-	98/122	104/92
2	136/150	232/200	276/A	120/119	-/-	130/122	104/104
3	136/146	—	276/A	120/119	-/-	98/118	104/104
4	150/150	226/200	B/A	120/119	-/+	98/122	—
5	136/146	232/200	276/272	120/119	-/-	130/122	104/104
6	150/150	226/200	B/A	120/119	-/+	98/122	104/104
7	150/150	—	B/A	119/119	-/+	98/122	—
8	136/150	—	276/A	119/119	-/-	130/118	—
9	136/146	232/200	276/272	119/119	-/-	130/122	104/104
10	150/146	232/200	B/272	119/119	-/-	130/118	104/104
11	150/150	226/200	B/A	119/119	-/-	98/118	104/104
12	150/146	232/200	B/272	119/119	-/+	98/118	104/104

* In progeny genotypes, the allele inherited from the female is listed first.

† For locus Pma-135, the larger allele in both parents is assigned a letter on the basis of relative size.

‡ For locus Pma-200, only one allele produced detectable PCR product.

male gametes is 15, giving a total of 30 tests. Both female and male gametes were in strong nonrandom association for the pair Pma-130/Pma-135 ($p = 0.0013$ and $p = 0.008$, respectively). We conclude that these two loci are linked. Indeed, we have observed only one recombinant gamete among a total of 24, which gives an estimate for recombination distance of 0.042, with an upper 95% limit of 0.124. The pair Pma-130/Pma-212 showed a weak nonrandom association among female gametes, but a random association among male gametes ($p = 0.042$ and $p = 0.538$, respectively). The same was observed for the pair Pma-135/Pma-212 ($p = 0.045$ and $p = 0.689$ for female and male gametes, respectively). The fact that in both cases the nonrandom association was observed among female gametes only is intriguing, but not statistically improbable. The result can be suggestive of linkage or of recombination suppression in only one parent (e.g., because of a chromosomal inversion), or it can be totally the result of random sampling.

Variation in a Natural Population

The data from the pair-mating suggest that, collectively, the seven loci could have sufficient discriminatory power to identify the parents of each progeny in a mass mating, provided the geno-

types of the parents are also known. To evaluate the parallel utility of these loci for studies of natural populations, we examined a sample from the commercially exploited scallop bed of Brown's Bank (Table 3). All loci were found to be polymorphic, but there were distinct differences among loci and most of these differences appear to be related to microsatellite core sequence. Observed number of alleles and expected heterozygosities were comparable among the four loci with a dinucleotide core; the allele length was continuously distributed within these loci, and the allele length range varied from 36–72 bp (or 18–36 repeat units). In contrast, observed alleles and heterozygosities varied widely in the three loci with compound core, allele length distribution was discontinuous, and the allele length range varied among loci from 10 to more than 500 bp.

Under the assumptions of selective neutrality of microsatellite variation and approximate Hardy-Weinberg equilibrium, the expected degree of heterozygosity and the number of observed alleles can be used to estimate the parameter $M = 4N_e u$. Two estimates (M_1 for the infinite alleles model and M_2 for the stepwise model) are given in Table 4. For loci with a dinucleotide core, the estimate of M based on the stepwise model (M_2) produced estimates that are about one order of magnitude larger than the esti-

TABLE 3.
Microsatellite variation in a population of *P. magellanicus*.

Locus	N	R	k_o	H_o	h (SE)	H_e	P_{hw}
Pma-130	51	114–186	24	47	0.9332 (0.010)	47.59	0.5385
Pma-200	37	134–182	19	24	0.9293 (0.010)	34.38	$p < 0.001$
Pma-212	53	86–154	26	36	0.9481 (0.007)	50.25	$p < 0.001$
Pma-275	55	88–124	18	50	0.9254 (0.008)	50.90	0.1499
Pma-132	47	192–246	12	16	0.5539 (0.060)	26.03	$p < 0.001$
Pma-135	54	252–780	30	43	0.9309 (0.009)	50.27	0.0465
Pma-180	54	109–120	6	21	0.3946 (0.052)	21.31	0.5085

N, sample size; R, size range of alleles in bp; k_o , observed number of alleles; H_o , observed number of heterozygotes; h , unbiased estimate of expected heterozygosity (standard error); H_e , expected number of heterozygotes; P_{hw} , probability for conformity to Hardy-Weinberg equilibrium.

TABLE 4.
Estimates of $M = 4 N_e u$.

Locus	M_1	M_2	M_2/M_1
Pma-130	13.97	111.55	7.98
Pma-200	13.14	99.47	7.57
Pma-212	18.27	185.17	10.14
Pma-275	12.40	89.28	7.20
Pma-132	1.24	2.01	1.62
Pma-135	13.47	104.20	7.74
Pma-180	0.65	0.86	1.33

N_e is effective population size and u is mutation rate for seven microsatellite loci under the assumption of selective neutrality. M_1 , infinite allele model; M_2 , stepwise mutation model. In both models, M was estimated from expected heterozygosity (see Text for details).

mates of the infinite allele model (M_1). The three loci with a complex core produce quite disparate estimates of M under either mutation model (Table 4), and the values of M_1 and M_2 are relatively similar to one another.

Genotype frequencies were found to be in conformity with Hardy-Weinberg expectations for three loci and to deviate at the other four. In all four cases, the deviation was in the direction of excess of homozygosity (Table 3). Excess of homozygosity for allozyme loci is a common phenomenon in natural populations of many species and in bivalves in particular (Zouros and Foltz 1984), but it is also known to occur in surveys of microsatellite variation (Pemberton et al. 1995, Ruzzante et al. 1996). This deviation from expectation from random mating may have different causes and implications for the use of microsatellites in population surveys (see below).

DISCUSSION

The wide, yet discontinuous distribution of *P. magellanicus*, its basically sedentary nature that makes local populations vulnerable to episodes of overfishing, and its passive yet potentially long-range dispersion though the planktonic larval stage make this species an interesting model for population genetics studies. Such studies are also of paramount importance for the management of this very important resource.

The seven microsatellite loci that we describe here present collectively the most useful genetic tool currently available for population studies in this species. Allozyme surveys produced little differentiation among local populations (Beaumont and Zouros 1991, Pogson and Zouros 1994), whereas nonspecific proteins appear to be less polymorphic (heterozygosity about 0.25; Pogson and Zouros 1994). In contrast, RAPD revealed 15 moderately polymorphic loci (Patwary et al. 1994). Thus, this source of variation, even though not high for any individual locus, can also be useful in large-scale population surveys, particularly because it is based on a simple and fast PCR assay. Restriction fragment-length polymorphisms recovered after hybridization to clones isolated from cDNA libraries provide another source of genetic markers in this species (Pogson 1994, Pogson and Zouros 1994). When the underlying cause of variation detected by cDNA probes is a variable number of tandem repeats, the amount of variation can be high (five such loci produced an average heterozygosity of 0.73; Pogson and Zouros 1994), but the variation is lower when the underlying cause is restriction site loss or gain (average heterozygosity of 0.27 on three loci; Pogson and Zouros 1994). However,

polymorphisms detected with cDNA clones cannot be amplified by PCR and are, therefore, not suitable for large-scale population studies. The mitochondrial genome of this species is also highly variable, but the variation is due to large-size repeats (Gjetvåg et al. 1992), which are not scorable by PCR. More important, this mtDNA variation has an extremely high rate of mutation (Cook and Zouros 1994), which makes it unsuitable for population discrimination or progeny testing studies. In contrast to these polymorphisms, the microsatellites that we have developed are easily scorable and are highly variable with an average heterozygosity of 0.8. Most of these loci can be scored in larvae and are, thus, ideal for monitoring the movement of larvae in wild or experimental populations (e.g., Manuel et al. 1996) or for experiments with broodstock designed to identify differences among genotypes for characters such mortality, settlement, and growth rates that could be of use in the development of the aquaculture of this species.

In order to obtain an indication of the amount of variation at each of the seven loci, we examined a sample from a natural population. This preliminary analysis revealed several patterns that might be of general interest for the use of microsatellites in population studies. Specifically, we have observed that several population parameters estimated from microsatellite scores, such as distribution of allele size, observed number of alleles, and expected heterozygosities, vary according to the physical properties of the loci examined. Although there were some differences with regard to the ease of scoring of loci with a dinucleotide repeat core (locus Pma-200 produces faint bands and can be scored reliably only in freshly extracted DNA), all four loci of this class produced comparable results with respect to the distribution of allele length, number of observed alleles, and degree of heterozygosity. One of the three loci with a complex core (Pma-135) produced a similar level of heterozygosity, but a higher number of observed alleles. In addition, the allele length distribution in this locus was distinctly bimodal, suggesting a stepwise mutation mechanism with two non-overlapping length ranges. The other two loci with a composite core had distinctively lower numbers of observed alleles and heterozygosities.

These differences between the two types of loci are reflected in the estimates of population parameters that can be obtained from the level of their polymorphism. One important question about the nature of microsatellite variation is the underlying mechanism of mutation (Shriver et al. 1993, Valdes et al. 1993, Weber and Wong 1993, Di Rienzo et al. 1994). The two most widely considered models are the infinite-alleles model, according to which any mutation event generates a variant that did not exist previously in the population, and the stepwise model, according to which an allele can only mutate to another that is smaller or larger by a defined number of nucleotide repeats. Allele frequency data cannot provide estimates of mutation rate. Instead, under certain assumptions, they can be used to obtain estimates of the parameter $M = 4N_e u$, that is, the product of mutation rate (u) and effective population size (N_e). The expected value of M under the infinite-alleles model is given by $M = n_e - 1$ (Kimura and Crow 1964) and under the stepwise model by $M = (n_e - 1)/2$, where $n_e = 1/(1 - h)$. Estimates of M for each locus under both models are given in Table 4. An important point is that four of the seven loci produce similar estimates (as seen from the ratio of M_2/M_1), a third locus (Pma-212) produces a somewhat larger estimate of mutation rate (assuming, as we must, that the effective population size is the same across loci), but two other loci produce a much smaller estimate. Both of these loci belong to the class with compound

core. This suggests that the mode of mutation may be different among microsatellite loci with a simple dinucleotide repeat and loci with a more complex core.

In four of the seven loci, genotype frequencies do not conform to Hardy-Weinberg expectation. Interestingly, these loci belong to all three classes with regard to the core sequence, suggesting that homozygosity excess is not related to core sequence. Excess of homozygosity can be real or apparent. Inbreeding, population mixing, and other patterns of nonrandom mating may cause a real excess, as can certain types of selection. Null alleles, alleles with incomplete penetrance, or differences in the intensity of allelic bands may cause an apparent excess of homozygosity. The difficulty of distinguishing between true and apparent homozygotes is a recurrent problem in the use of genetic markers for the study of natural populations, particularly of bivalves, where excess of homozygosity is a much more common phenomenon than is deficiency (see, for example, Gaffney 1994). As a result, it is often difficult to decide between explanations that are based on population structure and explanations that are based on physical properties of the markers used. The existence of genuine null alleles (Pemberton et al. 1995) may explain the excess of homozygosity at microsatellite loci. It is also possible that the PCR amplification rate varies among alleles, leading to an apparent absence of one allele and the incorrect scoring of a true heterozygote as a homozygote. Until these possibilities are excluded, it would be unwise to contribute the excess of homozygosity to mating population structure or selection.

In conclusion, the results we present here demonstrate that the microsatellite assays we have developed can be extremely useful for breeding experiments in laboratory or cultured populations of scallops. The multiallelic nature of the markers must also make them useful for the study of population structure and mixing under natural conditions and the related question of stock discrimination. However, the latter studies must take into consideration the possibility that microsatellites with different core sequences may provide different results with regard to various population parameters such as mutation rates (or effective population sizes), inbreeding, and population admixture. This variation of results among different microsatellite loci remains one of the most serious challenges in the use of microsatellites for population studies.

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EVALUATION OF HISTOLOGICAL CASSETTES AS HOLDING CONTAINERS FOR INDIVIDUAL SPAT, AND A WEEKLY HANDLING PROTOCOL TO ASSESS GROWTH OF THE SILVER-LIP PEARL OYSTER, *PINCTADA MAXIMA* (JAMESON)

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ABSTRACT The effects of holding *Pinctada maxima* spat within individual histological cassettes and of weekly handling on their growth, survival, and feeding were assessed by use of a flow-through culture method. Neither growth or survival was compromised by the use of the cassettes or by weekly handling. Initial spat size was not related to the subsequent specific growth rate; thus, rigorous grading is not essential in nursery experiments using *P. maxima*. Spat between 8.7 and 824 mg live weight had similar proportions of dry and ash-free dry weights of 63.5 and 5.5% of live weight, respectively. Daily algal consumption (dry weight of algae/live weight) ranged from 0.3 to 0.7%. Conversion efficiency for spat held in cassettes was higher (46.8%) than that for free spat (30.3%). The low algal consumption and high conversion efficiencies may reflect the oligotrophic environment in which *P. maxima* is naturally found.

KEY WORDS: pearl oyster, *Pinctada maxima*, feeding, bivalve, conversion efficiency

INTRODUCTION

The pearl industry in Australia was worth an estimated \$Aus200 (\$US140) million per annum in 1994 (Knuckey 1995). Although it has historically been reliant on the supply of wild oysters for pearl production, there is now a significant expansion of the use of hatchery-produced spat. Hatchery methods for *Pinctada maxima* developed in western Australia from 1987 to 1989 form the basis for current spat production in commercial hatcheries (Rose and Baker 1994). One of the areas identified as requiring further research is the nursery culture of spat, both in the hatchery and on the farm.

Recent literature has indicated that food concentration is an important aspect of feeding pearl oyster spat. Numaguchi (1994) determined that the optimal algal concentration for *Pinctada fucata* spat was 0.44 mg dry weight L^{-1} of *Pavlova lutheri*, equivalent to approximately 20 cells μL^{-1} . A concentration of 25 cells μL^{-1} of Tahitian *Isochrysis* sp. (T. Iso) was found to be the optimum for spat of the Indian strain of *P. fucata* (Krishnan and Alagarswami 1993). Results from scope-for-growth trials predicted an optimal cell density of approximately 17 cells μL^{-1} of T. Iso (0.34 mg L^{-1}) for *P. maxima* spat (Bellanger 1995). It therefore appears appropriate to feed pearl oyster spat by maintaining a food concentration of approximately 20 cells μL^{-1} in the water column. The use of flow-through systems allows the maintenance of a given algal concentration, in comparison to the more traditional approach of a ration that is fed once or twice per day within a static system.

There are very few reports in the literature where the performance of individual spat are monitored during nursery trials. This may be a reflection of bivalve spat generally being in plentiful supply, and relatively inexpensive compared with pearl oyster spat. Hence, the number of spat involved in these experiments is often large. Pearl oyster spat are difficult to grade because they have widely varying growth rates, attach to a substrate with a byssus, resettle quickly, and are of irregular shape. This makes it difficult to obtain similar-sized animals for experimentation.

To monitor individuals within a trial, each animal must be labeled in some manner, and this may present technical difficulties. Roegner (1990) settled *Crassostrea virginica* spat onto plates that were subsequently periodically photographed, and the shell growth of individual spat was determined by image analysis. This approach is not suitable for pearl oyster spat because they are motile and often only attach temporarily.

The use of histological cassettes as holding containers for individual spat within a replicate was evaluated. This technique would allow greater flexibility in the size range of spat that could be used in experiments, because the growth of individual spat could be monitored and the specific growth rate of individuals could be compared. This increases the statistical power of an experiment and allows the performance of individual animals to be assessed. Histological cassettes have previously been used in spat trials at the Darwin Aquaculture Centre (Tlili and Mills unpubl. data).

The effects of handling spat, for the purpose of monitoring growth, have rarely been investigated as a factor in bivalve research. Jakob and Wang (1994) concluded that handling *C. virginica* spat during nursery culture enhanced growth, although this result must be interpreted with caution because there was no replication. This experiment was conducted using a flow-through system to examine the effects of using histological cassettes as holding containers and of weekly growth monitoring on growth, survival, and feeding of *P. maxima* spat.

MATERIALS AND METHODS

Experimental Animals

Spat were produced by the Darwin Hatchery Project in May 1996 using western Australian broodstock and were held at the Bynoe Harbour Pearl Company farm until required. In August 1996, approximately 700 spat were transported to the Darwin Aquaculture Centre (Northern Territory Department of Primary Industry and Fisheries), packed between dampened cloth within an insulated box. Spat were acclimated for several days in a 500-L

tank at ambient temperature (24°C), during which time they were fed a mixed diet of *Isochrysis* sp. clone T. Iso (T. Iso), *Chaetoceros muelleri*, and locally isolated strains of *Tetraselmis* and *Cryptomonas* at a combined concentration of approximately 30 cells μL^{-1} . After acclimation, 200 spat were cleaned of biofouling and restocked into a 400-L recirculating system within an isothermal room at 24°C. The temperature of the room was subsequently increased to 28°C over 7 days. During this time, spat were fed a diet similar to that given during the acclimation period.

Shell dimensions and weights (live, dry, and ash-free dry weight), were determined for 30 randomly selected spat. The data were used to investigate the relationship between the measured variables within the initial size range used in the experiment (4.1–11.5 mm shell height, 8.7–173 mg).

Experimental System

The experimental system used was based on a flow-through method, the volume of which was controlled by fixed-flow irrigation drippers (Fig. 1). Spat were maintained either free (F) or in histological cassettes (C) (Fig. 2) on rigid plastic mesh within an aerated 1-L plastic tray (Fig. 3). Spat were continuously fed a suspension of T. Iso and *C. muelleri* (Taylor et al. 1997, Southgate and Sanders unpubl.) from a dedicated reservoir at a combined concentration of 1 mg L^{-1} . The mean total volume delivered over 24 h to each replicate by a submersible pump (Sacem Zephyr 100) and 21-h irrigation drippers (Philmac Pty. Ltd.) was 12.3 ± 0.7 L. The positioning of the aerator and the algal inlet ensured maximum mixing of the algal suspension within the tray. The contents of the reservoirs were replaced daily with fresh algae and carbon-filtered (1- μm -pore-size filter) seawater. Trays and drippers were replaced weekly to prevent fouling.

There were 10 replicates for each holding method, each containing six pearl oyster spat. Five of the replicates from each holding method were handled weekly (H) and live weight, shell height, and hinge length were measured, while the remaining five were not measured (U) during the 3-wk duration of the experiment. Shell height was taken as the distance from the hinge to the shell margin between the shell processes. Oysters were first cleaned with a soft brush and then blotted dry with a paper towel before being weighed to the nearest 0.1 mg.

Algal consumption for each replicate was determined by collecting all of the effluent passing through the trays during the 24 h before spat measurement. A 50-mL sample of the collected efflu-

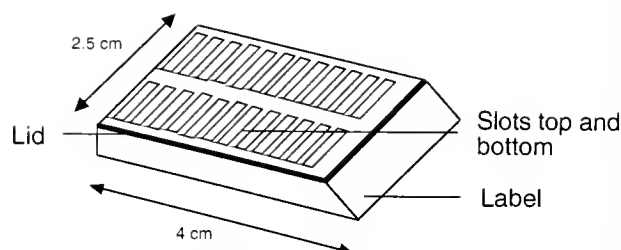


Figure 2. Histological cassette.

ent was preserved with 1% w/v iodine solution, and the residual algae were counted with a hemocytometer at $\times 400$ magnification. Replicate control systems ($n = 3$) containing no spat confirmed that there was no algal loss due to sedimentation within the system. Typical effluent algal concentrations were between 0.3 and 0.6 mg L^{-1} . Individual dry cell weights used to calculate algal dry biomass concentrations for T. Iso (19 pg) and *C. muelleri* (20 pg) were those of Nell and O'Connor (1991). Temperature was maintained at $28 \pm 0.5^\circ\text{C}$; dissolved oxygen saturation levels ranged from 93 to 100%, pH ranged from 8.12 to 8.23, and salinity ranged from 35 to 37‰.

The weight-specific algal consumption (C) for each replicate (dry weight of algae consumed as % of live spat weight over 24 h) was calculated by the equation:

$$C = (I - F) \cdot V / W \cdot 100 \quad (1)$$

where I is initial algal concentration (mg L^{-1} dry weight), F is final algal concentration (mg L^{-1} dry weight), V is volume of effluent (L), and W is live weight of spat in the replicate (mg).

Specific growth rate (% increase/day) on the basis of live weight (WSGR) and shell height (SSGR) was calculated using the formula of De Silva and Anderson (1995).

$$\text{SGR} = \frac{\ln(W_{t_2}) - \ln(W_{t_1})}{t_2 - t_1} \cdot 100 \quad (2)$$

Gross conversion efficiency was calculated (modified from De Silva and Anderson 1995) as

$$\text{WSGR} / C \cdot 100 / \text{organic \%} \quad (3)$$

Dry weights of spat were obtained after drying to constant weight at 103°C for 24 h. The organic content of individual spat was determined by subtraction after ashing at 475°C for 5 h in predried and weighed 5-mL porcelain crucibles.

Statistical Analysis

Weight-specific growth rate (WSGR), survival, and algal consumption were examined using a two-way analysis of variance model with handling and holding method as the two factors. Survival percentage data were arcsine $\sqrt{}$ transformed before being

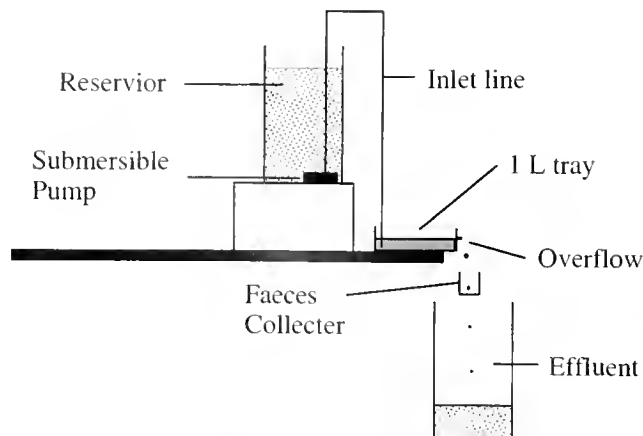


Figure 1. Flow-through spat experimental system.

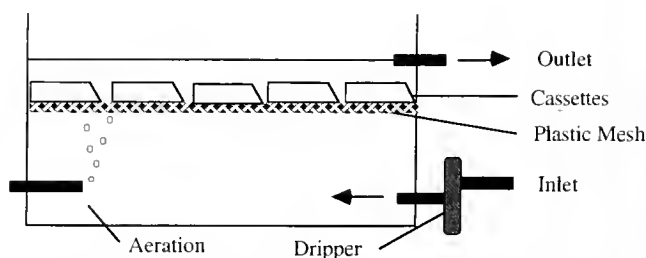


Figure 3. Details of the plastic trays used to maintain spat.

analyzed. Homogeneity of variances was confirmed with Cochran's test, and normal distributions were confirmed with the Shapiro-Wilk W test. The relative proportions of dry weight and organic content were compared with those of control oysters using Dunnett's test. Relationships between various spat measurements were examined using linear regression analysis of log-transformed data. A p value ≤ 0.05 was considered significant.

RESULTS

There was no effect or interaction of holding method or handling on either growth or survival of *P. maxima* spat (Table 1). Survival was generally high, although there was total mortality in one of the cassettes unhandled replicates, which was excluded from the analysis because it was not considered to be consistent with a treatment effect. There was no significant relationship between the initial live weight of individual spat and their subsequent specific growth rate during the experiment ($r = -0.36$; Fig. 4), indicating that no bias was introduced by the use of different sizes at the beginning of the experiment spat in the experiment. Treatment spat did not differ significantly from initial spat in the proportions of either dry weight or organic content (Dunnett's test, $p > 0.05$). Dry weight comprised $63.5 \pm 0.03\%$ of live weight. Organics comprised $5.5 \pm 0.09\%$ of live weight and $8.7 \pm 0.09\%$ of dry weight, respectively ($n = 130$). There were no size-related differences in the proportions of dry weight or organic content within the size range used in this experiment.

There was a significant reduction in algal consumption ($p < 0.05$) in all of the treatments during the experiment, with a corresponding decline in growth rate (Fig. 5). Although there were no differences in growth between free spat or those held in cassettes, free spat consumed a significantly greater amount of algae in relation to their live weight. Algal consumption (dry weight of algae:live weight) by free spat was $0.46 \pm 0.04\%$ compared with $0.32 \pm 0.04\%$ for spat held in cassettes. Conversion efficiencies were 46.8% for spat held in cassettes and 30.3% for free spat. The trends in growth and algal consumption were evident after only 7 days of culture.

There was a weak, though significant, relationship between the algal consumption of a replicate and its subsequent WSGR ($r^2 = 0.53$, $p = 0.003$). There were strong correlations between the weight indices and shell dimensions of spat, with shell length being a better indicator of live weight than was hinge length ($r^2 = 0.88$ and 0.92 , respectively). The relationship between shell height and live weight can be described by the regression model: Live weight = 0.00136 shell height.^{2.02}

DISCUSSION

There appears to be no detrimental effect of using histological cassettes as holding containers in *P. maxima* spat culture trials.

TABLE 1.

Growth (live weight) and survival of *P. maxima* spat in relation to holding method and handling frequency.

Treatment	Growth (WSGR %/day \pm SE)	Survival (% \pm SE)
Cassettes + handling	2.9 (0.1) ^{a*}	96.6 (3.4) ^a
Free + handling	2.8 (0.2) ^a	80.0 (6.1) ^a
Cassettes unhandled	2.6 (0.3) ^a	87.3 (7.3) ^a
Free unhandled	2.8 (0.2) ^a	90.0 (10.0) ^a

* Values with similar superscripts are not significantly different ($p > 0.05$).

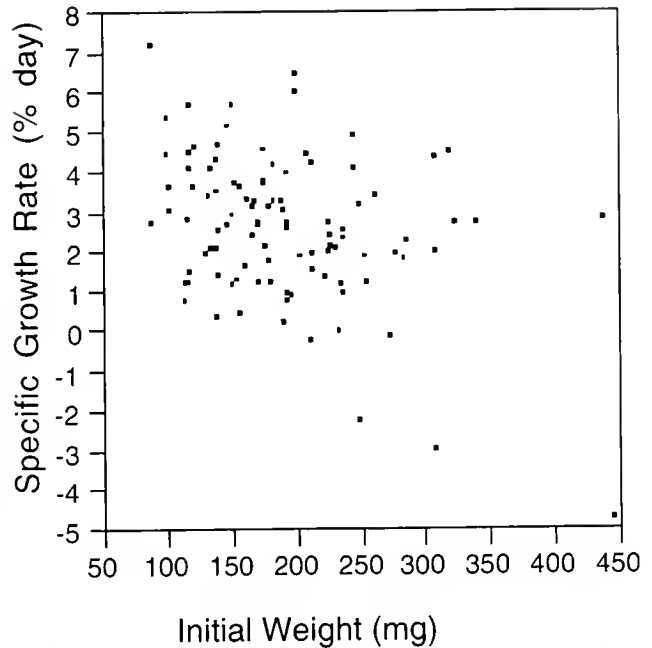


Figure 4. Scatterplot of initial spat size and subsequent specific growth rate (% increase/day) ($n = 130$).

Survival and growth rates were generally high and comparable with those obtained by Taylor et al. (1997) and Southgate and Sanders (unpubl.) in feeding trials (Table 2).

The growth rates shown in Table 2 indicate that *P. maxima* spat are relatively slow growing compared with those of *P. fucata* and *Pinctada margaritifera* and that spat held in farm-based nurseries grow faster than those maintained in the hatchery. This is presumably related to differences in nutrition and/or water quality and indicates that the hatchery environment and diets currently provided for pearl oyster spat need further optimization. The WSGR of pearl oyster spat is generally lower than that of other bivalve spat, which is commonly 5 to 11%/day (Laing and Millican 1991, Curatolo et al. 1993, Coutteau et al. 1994).

Over the initial size range of spat used in this experiment, there was no effect of initial size on the subsequent specific growth rate. This indicates that stringent grading is not essential, provided that

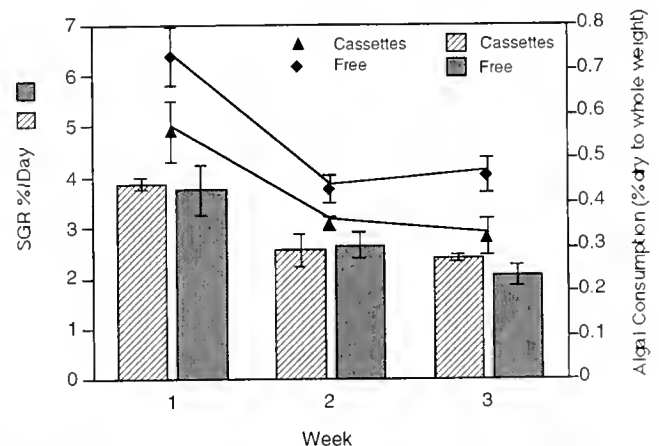


Figure 5. Growth and algal consumption over successive weeks of *P. maxima* spat held in histological cassettes or free on plastic mesh.

TABLE 2.
Growth of pear oyster spat in nursery trials.

Species	Growth (WSGR)*	Growth (SHSGR)†	Reference
<i>P. maxima</i>	2.65	0.97	Present study
<i>P. maxima</i>	1.7–5.1	0.17–1.24	Taylor et al. (1997)
<i>P. maxima</i>	2.43–3.6	0.9–1.13	Southgate and Sanders, unpubl.
<i>P. maxima</i>	3.5‡	1.39‡,§	Rose and Baker (1994)
<i>P. maxima</i>	ND	1.9‡,§	Tanaka and Kumeta (1981)
<i>P. fucata</i>	ND	1.2–5.1	Numaguchi (1994)
<i>P. fucata</i>	ND	0.17–0.7	Okauchi (1990)
<i>P. fucata</i>	ND	3.82	Alagarwami et al. (1983)
<i>P. margaritifera</i>	ND	3.88‡	Alagarwami et al. (1989)

* Live weight specific growth rate %/day.

† Shell height specific growth rate %/day.

‡ Farm-held spat.

§ Estimated.

|| ND, no data.

the performance of individual spat can be monitored. This aspect of growth provides several benefits. Constantly resettling spat during grading may be deleterious and may lead to more or less compromised spat being used; thus, repetitive grading to obtain a pool of similar-sized animals is undesirable. Experiments that involve temperature or diet may incorporate an acclimation period. After acclimation, spat sizes will cover a range reflecting the different treatments. However, this is less of a problem when spat of different initial sizes can be used without introducing any bias.

The lack of a relationship between initial size and WSGR also suggests that the difference in spat growth is not genetically predetermined, but is a result of environment and/or husbandry practices. This indicates that factors such as density and spat micro-environment may be critical in spat culture.

The reason for the higher growth in the first week of culture is unclear. It is possible that this growth was primarily shell rather than tissue growth. However, this is unlikely, because the greater growth was reflected by higher algal consumption, which suggests that the increase was allometric, as indicated by the correlation between algal consumption and WSGR. A similar burst of growth has been shown by adult oysters when initially placed into brood-stock conditioning trials (D. Mills, unpubl.). Pearl farmers also report that any significant disturbance to oysters, such as cleaning, stimulates growth. The initial growth burst may be a response to the stress of stocking into the experimental system.

Alternatively, the higher initial growth rate of the spat may have been a reflection of the diet they were being fed before being stocked into the experimental system. This consisted of a mixture of four species, and this diet promoted better growth than the two-species mixture used in this experiment. There may have been a short-term flow-on effect on growth during the first week of the experiment.

An advantage of the use of a flow-through system is that the feeding rate is to a large degree self-compensating for spat growth, although this will be related to the rate of exchange. This contrasts with static systems, where the effective feeding rate will change according to the increase in biomass, such as in the study by Southgate and Sanders (unpubl.), where the initial ration was 0.47% (dry weight of algae to live weight), but after 35 days, the effective rate was only 0.17%, because of the increase in spat biomass. Taylor et al. (1997) attempted to compensate for growth

by sequentially increasing the ration; however, at the end of the experiment, the effective feeding rates for different treatments ranged from 1.2 to almost 2% because of the differences in final biomass in different treatments. Thus, when using the batch-fed static system, there is often an experimental bias introduced. Another aspect of batch feeding in static systems is that the spat may be exposed to their optimal food concentration for only a small percentage of the feeding cycle. Numaguchi (1994) found that the grazing rate of *P. fucata* spat was 2.4% (dry weight of algae to live weight) at the optimal algal density, increasing to 3.8% at higher concentrations, although growth was not enhanced. This suggests that the extra algae grazed was not assimilated. A similar pattern has been shown for *P. maxima* spat by Bellanger (1995), where scope for growth was maximal at 17 cells μL^{-1} of T. Iso, and declined with increasing or decreasing algal concentration. Thus, in static systems, the initial high concentrations may be poorly utilized and even repress growth, whereas at the end of the feeding period, the food concentration may be too low to support maximum growth.

Coutteau et al. (1994) calculated the WSGR for clam spat and used this to compensate daily for increased spat biomass. Although this approach may allow a better maintenance of a ration based on the biomass of spat within a replicate, it does not overcome the problem of changes in food concentration during the feeding period, and in that experiment, diurnal algal concentrations within replicates varied from 15 to 130 cells μL^{-1} .

P. maxima spat, being essentially oceanic, may be less pre-adapted to large fluctuations in algal density compared with bivalves that inhabit more dynamic environments, such as intertidal zones and estuaries. Flow-through systems also allow the maintenance of higher water quality due by continual flushing.

The rate of algal consumption in this study (0.3–0.7% dry weight of algae to live weight day^{-1}) for *P. maxima* is very similar to that obtained by Bellanger (1995) of 0.46–0.675% (Table 3). In the same study, the consumption rate for *Pinctada albina* was found to be 0.8%. Southgate and Sanders (unpubl.) exposed *P. maxima* spat to rations of between 0.17 and 0.47% and achieved

TABLE 3.
Comparative algal consumption rates of some bivalve spat.

Species	DTW %*	Consumption†	Reference
<i>P. maxima</i>	8.7	3.5–8.0	This study
<i>P. maxima</i>	8.7‡	4.6–7.4	Bellanger (1995)
<i>P. albina</i>	8.7‡	8.8	Bellanger (1995)
<i>P. fucata</i>	8.7‡	26.4	Numaguchi (1994)
<i>Tapes philippinarum</i>	14.0	16.7	Laing and Verdugo (1991)
<i>M. mercenaria</i>	10.3	25.8	Laing and Verdugo (1991)
<i>Tapes decussata</i>	15.7	16.8	Laing and Verdugo (1991)
<i>Crassostrea gigas</i>	9.2	26.7	Laing and Verdugo (1991)
<i>M. mercenaria</i>	10.2§	19.5	Coutteau et al. (1994)
<i>Tapes philippinarum</i>	14.0§	20.4	Laing and Millican (1991)

* Dry tissue weight/live weight.

† Dry algal weight: dry tissue weight.

‡ Dry tissue weight % assumed to be the same as in this study.

§ Dry tissue weight % assumed to be the same as in Laing and Verdugo (1991).

growth rates similar to those of this study. Okauchi (1990) exposed *P. fucata* spat to rations ranging from 0.3 to 0.6%, well below the 2.4% optimum found by Numaguchi (1994). This may have accounted for the lower growth achieved in this study (Table 2). *P. fucata* appears to be adapted to more eutrophic conditions than is *P. maxima*, as shown by the higher algal consumption, which is similar to that of *Mercenaria mercenaria* of 2.5% (Coutteau et al. 1994) and 2.86% (Laing and Millican 1991). The generally low algal consumption relative to live weight for pearl oyster spat may be partly a function of shape, which is quite laterally compressed when compared with that of other bivalves. This results in a greater shell-to-tissue ratio and hence a lower proportion of tissue to live weight (Table 3). The consumed ration may be better described as a function of the algae consumed relative to dry tissue weight, although this shows that *P. maxima* spat still have a relatively low feeding rate compared with that of other bivalves (Table 3).

The lower feeding rate of *P. maxima* spat is probably a reflection of the relatively oligotrophic waters that it generally inhabits. This is also reflected in the mean conversion efficiency of 38.6% compared with that of approximately 20% for *M. mercenaria* (Laing and Millican 1991).

The relative proportion of organic content to live weight was constant over the range of sizes used in the experiment. The mean organic content (5.5%) was within the range obtained by Southgate and Sanders (unpubl.) of 4.4–6%, but lower than that reported for

Indonesian spat by Taylor et al. (1997) of 6–8.2%. This may indicate that there are genetic differences in the shell:meat ratio between the Australian stocks and those from Indonesia or may result from differences in culture methods. The spat used by Taylor et al. (1997) had an initial organic content of only 4.47%, which is very low compared with the final range and is even lower than that of the unfed controls at the completion of the experiment (4.86%). This low initial value is similar to that obtained by Southgate and Sanders (unpubl.) for starved spat (3.9%).

The results of Taylor et al. (1997) show a strong positive relationship between the organic content and WSGR of *P. maxima* spat fed various algal diets ($r = 0.77$). This suggests that the organic content may be a good indicator of the nutritional status of spat. However, no such relationship was shown in this study or in that of Southgate and Sanders (unpubl.).

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HATCHERY AND EARLY NURSERY CULTURE OF THE BLACKLIP PEARL OYSTER (*PINCTADA MARGARITIFERA* L.)

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ABSTRACT This article reports on spawning induction and larval and early nursery culture of the blacklip pearl oyster *Pinctada margaritifera* (L.). Spawning was induced using thermal "shock," where water temperature was manipulated from an overnight low of 22°C to a high at spawning of 32–33°C. Larvae were cultured in 500-L tanks in which the water was replaced every 3–4 days (static system) or in 500-L flow-through tanks in which 100% of the tank water was changed every 24 h. There was no significant difference in survival or growth of the larvae in static or flow-through tanks. Mean (\pm SE) anteroposterior shell length (APM) on Day 20, when larvae were transferred to settlement tanks, was 214.38 (\pm 3.06) μ m and 217.52 (\pm 2.93) μ m for static culture and flow-through culture tanks, respectively. Spat held in settlement tanks had a mean (\pm SE) dorsoventral shell height (DVH) of 1.38 (\pm 0.03) mm at 43 days postfertilization when they were placed in plastic mesh trays and transferred to the sea. At 106 days of age, spat were removed from collectors and graded. The mean (\pm SE) DVH of 106-day-old spat was 11.2 (\pm 2.7) mm; the largest individual had a DVH of 23 mm, whereas the smallest was less than 2 mm. At grading, 0.2, 8.9, and 67.3% of spat were retained on 15-, 10-, and 5-mm plastic mesh, respectively, and 23.6% fell through the 5-mm mesh. Growth of spat in plastic trays and pearl nets was assessed at densities of 10, 50, and 100 per tray and at densities of 20, 50, 100, 150, and 200 per net over a 19-wk growth trial. DVH was significantly greater in pearl oysters held in plastic trays at a density of 100 per tray (40.48 \pm 0.9 mm). Oysters held at this density also had the greatest APM (39.68 \pm 0.9 mm) and wet weight (7.44 \pm 0.4 g). Pearl oysters held in pearl nets showed the greatest DVH (39.22 \pm 0.6 mm), APM (38.36 \pm 0.6 mm), hinge length (34.47 \pm 0.5 mm), and wet weight (6.84 \pm 0.8 g) at the lowest density of 20 per net. These values did not differ significantly from those of juveniles held at a density of 50 per net. Growth of juveniles held at densities of 20 and 50 per net was significantly greater than that of juveniles held at densities of 100, 150, and 200 per net. The presence of leatherjackets (*Paramonacanthus japonicus*) in trays and nets significantly affected growth rates of the spat.

KEY WORDS: pearl oyster, spawning, larvae, spat, growth, survival

INTRODUCTION

Pearl culture has traditionally relied on the collection of pearl oysters from the wild. Oysters are either collected as adults or collected as spat that are on-grown to a size suitable for pearl production. In the Pacific, the blacklip pearl oyster (*Pinctada margaritifera* L.) supports well-established cultured pearl industries in French Polynesia and the Cook Islands. The former generated an estimated income of US \$135.3 million in 1994, while the value of the Cook Islands industry was estimated US \$4.5 million in 1993 (Fassler 1995). Not surprisingly, there is considerable interest from other Pacific nations in developing similar cultured pearl industries. In a number of countries, however, such development is prevented by low natural stocks of pearl oysters (Southgate 1995, Southgate 1996). Clearly, for countries with low stocks of adult pearl oysters, the opportunity to develop a cultured pearl industry based on wild spat collection is very limited and development is only likely using hatchery-produced seed. Recent years have seen the development of hatchery techniques for pearl oysters (Alagar-swami et al. 1983, Alagar-swami et al. 1989, Rose and Baker 1994) and an increasing use of hatchery-produced seed in culture operations (Gervis and Sims 1992). Hatchery production of *P. margaritifera* seed is limited, and difficulties have been encountered (Coeroli et al. 1984). Nevertheless, Alagar-swami et al. (1989) reported successful experimental hatchery production of *P. margaritifera* in India, and commercial seed production now occurs in French Polynesia and Okinawa (Sims 1993) and in Hawaii (Clarke et al. 1996).

Information on the hatchery rearing of pearl oysters is limited, although the techniques used are similar to those developed for other bivalves. Larvae are usually reared in static culture tanks with periodic water changes (Alagar-swami et al. 1989, Gervis and

Sims 1992, Rose and Baker 1994). Southgate and Ito (in press) recently described a flow-through larval culture system used successfully for *P. margaritifera*; however, this system has not yet been evaluated against a conventional static culture system. Although suitable hatchery techniques are becoming established and experimentally evaluated for *P. margaritifera*, very little information is available on appropriate methods for nursery culture of hatchery-produced spat. This results primarily from the traditional use of wild-collected *P. margaritifera* spat as the basis for cultured pearl industries in the Pacific (Coeroli et al. 1984, Gervis and Sims 1992, Friedman and Bell 1996). Spat collected in this manner are generally left on collectors for approximately 6 mo before being transferred to juvenile culture systems (Gervis and Sims 1992). As such, until the relatively recent interest in hatchery production of *P. margaritifera*, there has been no incentive to establish nursery culture techniques for young spat and juveniles. This article reports on the successful production of *P. margaritifera* seed in Australia and on the evaluation of novel hatchery and nursery techniques.

MATERIALS AND METHODS

Spawning Induction

Adult *P. margaritifera* were held in eight-pocket panel nets (Gervis and Sims 1992) suspended from a longline at a depth of 3–4 m at Pioneer Bay, Orpheus Island, North Queensland, Australia (latitude, 18°35'S; longitude, 146°29'E). Broodstock were removed from the longline, scrubbed, and washed with filtered (1- μ m-pore-size filter) seawater (FSW) to remove sediment and fouling organisms. Cleaned broodstock were placed upright in plastic aquaria containing a minimum volume of FSW and held overnight in an air-conditioned room with an air temperature of

22°C. The following morning, broodstock were placed into a shallow raceway containing only sufficient FSW to just cover the oysters. Spawning was induced by thermal stimulation; before the introduction of broodstock to the raceway, the temperature of the raceway water was raised to around 30–32°C with water heaters or by the addition of heated FSW. Spawning oysters were removed from the raceway into individual containers and allowed to complete spawning. Fertilized eggs were collected on a 25- μm -pore-size mesh screen and washed briefly with FSW. Eggs were incubated in gently aerated 500-L tanks containing FSW at a density of 30–50 mL^{-1} (Southgate et al., in press). After 24 h, D-stage veliger larvae were removed from the incubation tank onto a 25- μm -pore-size mesh screen, counted, and placed into larval rearing tanks.

Larval Rearing

Six outdoor 500-L tanks were filled with FSW, and each was stocked (on Day 1) with 1-day-old *P. margaritifera* veligers at a density of 2 mL^{-1} . Three of the tanks were run using static culture conditions and were provided with gentle aeration. Static tanks were drained, washed, and refilled with clean FSW on Days 4, 7, 11, 14, and 17. Larvae from each tank were removed onto a mesh screen and held in a 20-L bucket containing fresh seawater before being returned to the tanks. The remaining three tanks were set up as flow-through tanks as described by Southgate and Ito (in press). Each tank was provided with a central standpipe to which a mesh cone and float were attached (Fig. 1). The mesh allowed a through-flow of water but prevented escape of the larvae. The pore size of the mesh was increased from 37 μm to 53 μm and finally to 74

μm , on Days 8 and 15, respectively. Water passed through the flow-through tanks for 12 h/day at a flow rate sufficient to replace 100% of the tank volume during this period. The flow-through tanks were completely drained and washed on Days 8 and 15, and the larvae were retained as described above. Water temperature was measured in each tank at 09:00 and 21:00 each day. Water samples were removed on Days 7 and 20 from both static and flow-through tanks for analysis of ammonia and nitrite content by the methods outlined by Franson (1995). Water samples from static-culture tanks were removed immediately before water change. Water temperature in the static and flow-through tanks ranged from 26.3 to 30.1°C and from 26.5 to 30.1°C, respectively, during the larval culture period.

Larvae were fed a mixed diet of cultured microalgae consisting of *Isochrysis* aff. *galbana* clone T-ISO (CS 177), *Chaetoceros simplex* (CS 251), and *Pavlova salina* (CS 49). All three species are well suited for use in tropical conditions (Jeffrey et al. 1992). Starter cultures were obtained from the CSIRO Fisheries Division in Hobart, Tasmania, and the codes above refer to CSIRO catalogue codes. Microalgae were initially cultured in 3- to 5-L glass flasks in filtered (0.45 μm pore size) and ultraviolet-treated seawater with the nutrient medium described by Southgate and Ito (in press). Larger culture volumes were maintained in 30-L plastic tubs. All algae were fed to larvae and spat during the exponential growth phase. The feeding rate for larvae is shown in Table 1.

Settlement and Nursery Culture

On Day 20, eyed larvae large enough to be retained on a 150- μm -pore-size mesh screen were removed from the larval culture tanks and placed into 500-L settlement tanks. Each settlement tank contained FSW vigorously aerated with five air lines. Seventy-five spat collectors were suspended in each settlement tank. Each collector measured approximately 30 \times 15 cm and consisted of an outer "onion" bag filled with approximately 0.5 m^2 of 50% woven shade cloth.

Cultured microalgae were added to the settlement tanks at the rates shown in Table 1. Water in the settlement tanks was completely exchanged on a daily basis using a flow-through system, and water temperature ranged from 26.5 to 30.1°C during the study. On Day 43, spat collectors were removed from the settle-

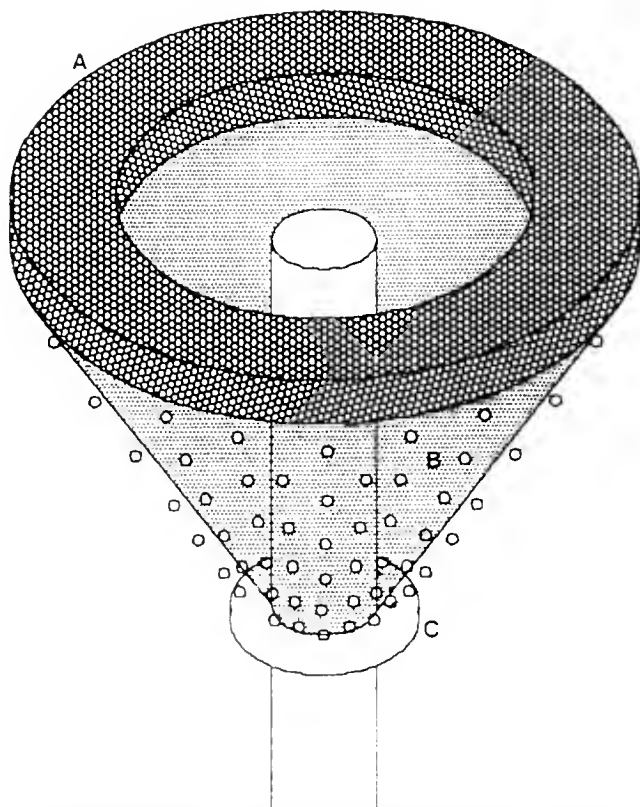


Figure 1. Apparatus placed onto central stand pipe in "flow-through" culture tanks used for *P. margaritifera* larvae. A = float, B = mesh cone, and C = flexible aeration tubing.

TABLE 1.

Feeding rates for *P. margaritifera* larvae and spat; larvae were initially stocked at a density of 2 mL^{-1} , and larvae were removed into settlement tanks on Day 20.

Day	Feeding Rate (Cells mL^{-1})
1–4	1,000
5–7	2,000
8	4,000
9–12	8,000
13–14	10,000
15–19	12,000
20–25	10,000
26–27	12,000
28–29	18,000
30–34	25,000
35–39	30,000
40–43	35,000

ment tanks and placed inside plastic mesh trays (55 × 30 × 10 cm) with lids; four collectors were tied into each tray, and trays were then weighted and suspended from a surface longline at a depth of 6 m. On Day 106, 63 days after being placed into the sea, spat were removed from the collectors and graded through plastic mesh screens. Spat that passed through a 15-mm-pore-size (square) mesh and were retained on a 10-mm-pore-size mesh were placed into the same plastic mesh trays used for housing spat collectors at densities of 10, 50, and 100 per tray, and plastic mesh lids were placed onto the trays. Three replicate trays of each stocking density were then suspended from a surface longline at a depth of 4 m. Spat that passed through the 10-mm-pore-size mesh but were retained on a 5-mm-pore-size mesh were placed into square-based pyramidal pearl nets (see Gervis and Sims 1992) made of 7-mm-pore-size nylon mesh; the sides of the base of the nets were 35 cm. Spat were stocked into pearl nets at densities of 20, 50, 100, 150, and 200 per net. Five replicates of each density were suspended from the longline at a depth of 4 m. At the start of the nursery growth trial, the mean (\pm SE) dorsoventral height (DVH) of individuals in crates and pearl nets was 13.9 ± 0.28 and 9.8 ± 0.24 mm, respectively.

Trays and pearl nets were brushed in situ approximately every 4 wk to reduce fouling. After 19 wk, juveniles were removed from the trays and pearl nets and counted; shell growth was measured as DVH, anteroposterior measurement (APM), and hinge length (HL) (see Fig. 2). All remaining juveniles in the 10, 20, and 50 treatments were weighed and measured, and 50 randomly selected juveniles were measured from the 100, 150, and 200 treatments. Survival data (%) were arcsin transformed before analysis. Data were analyzed using one-way analysis of variance, and significant differences between means were identified using the Tukey test (Zar 1984).

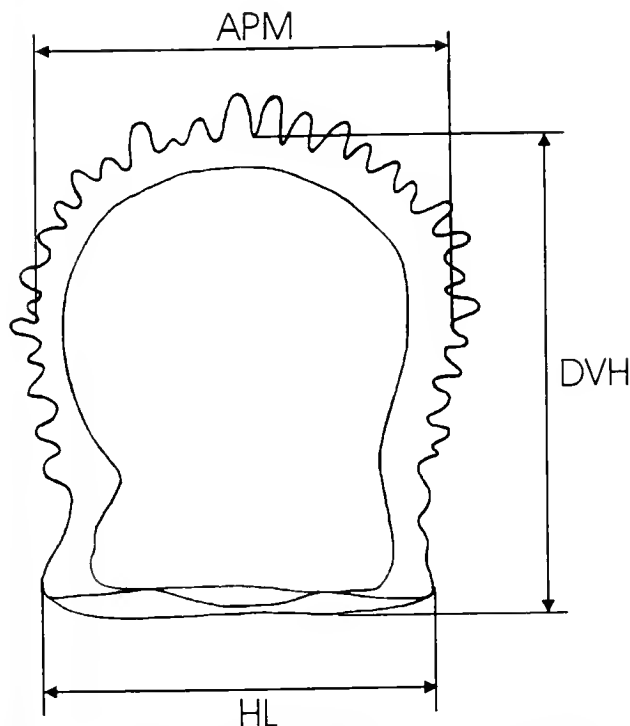


Figure 2. Dimensions used for measurement of *P. margaritifera* spat and juveniles.

RESULTS

Larval Development

Fertilized eggs had a mean diameter of 61.03 ± 1.04 μ m (\pm SE, $n = 30$). Development of *P. margaritifera* larvae was similar to that described by Alagarwami et al. (1989) and to that described for *Pinctada maxima* by Rose and Baker (1994). Changes in mean APM of *P. margaritifera* larvae cultured in static and flow-through culture tanks are shown in Figure 3. Larvae had reached the D-stage by 20–24 h after fertilization and had a mean APM of 82.09 ± 1.37 μ m. Umbonal larvae were first seen on Day 9; however, the majority of the larvae were umbonal on Day 11 when the mean APM was 138.28 ± 2.31 μ m. Growth rates were similar in both the static and the flow-through systems (Fig. 3). On Day 20, larvae from the static and flow-through systems had mean APM of 214.38 ± 3.06 and 217.52 ± 2.93 μ m, respectively. The relationship between APM (y) and DVH (x) is described by the equation: $y = 1.017x + 13.712$.

There was no significant difference between treatment in survival to Day 20 ($p = 0.842$). Mean (\pm SE, $n = 3$) survival to Day 20 was 4.33% (± 2.1) and 5.75% (± 3.8) in the static and flow-through tanks, respectively. Survival was very variable between replicate tanks of the same treatment and, for example, ranged from 1.9 to 9.6% in flow-through tanks. The mean proportion of the total number of larvae surviving to Day 20 that were large enough to be caught on a 150- μ m-pore-size sieve mesh was 52.1 (± 3.5)% in the static tanks and 63.0 (± 7.7)% in the flow-through tanks. These values did not differ significantly ($p = 0.328$).

Water Chemistry

Ammonia and nitrite levels in the static and flow-through tanks are shown in Table 2. There was no significant difference in the levels of ammonia or nitrite between static and flow-through tanks on Day 7. On Day 20, mean ammonia and nitrite levels were higher in both static and flow-through tanks than on Day 7; the

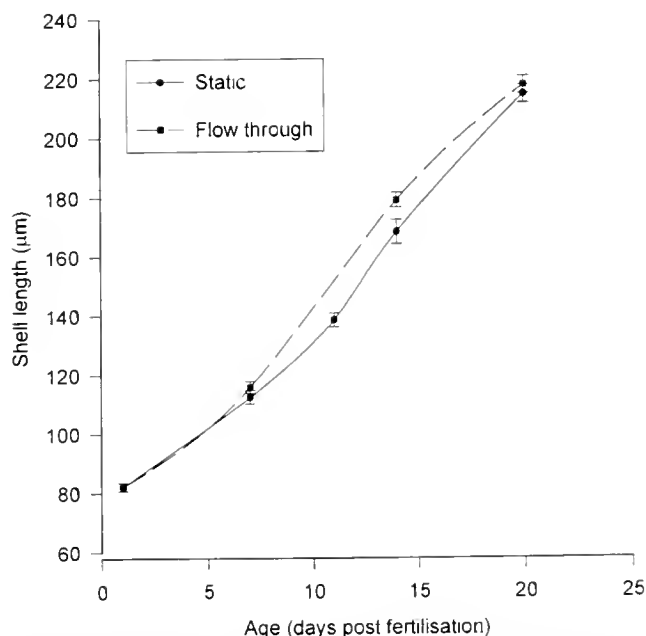


Figure 3. Changes in mean (\pm SE) anteroposterior shell length of *P. margaritifera* larvae cultured in flow-through (■) and static (●) tanks.

TABLE 2.

Ammonia and nitrite contents (mg L^{-1}) in seawater from static and flow-through larval culture tanks.

Day	Flow-Through Tanks		Static Tanks	
	Ammonia	Nitrite	Ammonia	Nitrite
7	13.23 ^a (± 0.69)	1.73 ^b (± 0.38)	15.57 ^a (± 0.87)	2.17 ^b (± 0.18)
20	18.73 ^c (± 0.18)	1.90 ^d (± 1.0)	27.20 ^c (± 1.3)	2.23 ^d (± 0.18)

Values are mean (\pm SE) from three determination (one from each replicate tank). Means for the same parameter within the same row with the same superscript are not significantly different ($p > 0.05$).

mean ammonia level in static culture tanks was $27.2 \pm 1.3 \text{ mg L}^{-1}$, and this was significantly higher than the mean ammonia level in the flow-through tanks of $18.7 \pm 0.2 \text{ mg L}^{-1}$ ($p = 0.018$). The mean nitrite level in static culture tanks was $2.2 \pm 0.2 \text{ mg L}^{-1}$ on Day 20, which was higher than the mean nitrite level in flow-through tanks of $1.9 \pm 1.0 \text{ mg L}^{-1}$; however, this difference was not significant ($p = 0.22$).

Spat Growth

Mean (\pm SE, $n = 50$) DVH of spat removed from the settlement tanks 43 days after fertilization was $1.37 \pm 0.1 \text{ mm}$. Mortality of larvae and spat in the settlement tanks was relatively high, and approximately 17% of the larvae placed into settlement tanks on Day 20 survived to Day 43. Mean monthly water temperature during the nursery study ranged from $28.4 (\pm 0.3)^\circ\text{C}$ at the start of the experiment in December to $23.7 (\pm 0.3)^\circ\text{C}$ at the end of the experiment in June; however, the highest water temperature of $29.8 (\pm 0.2)^\circ\text{C}$ was reached in February (Fig. 4). Growth of spat to grading at 106 days is shown in Figure 5.

The development of growth processes on the shell was evident

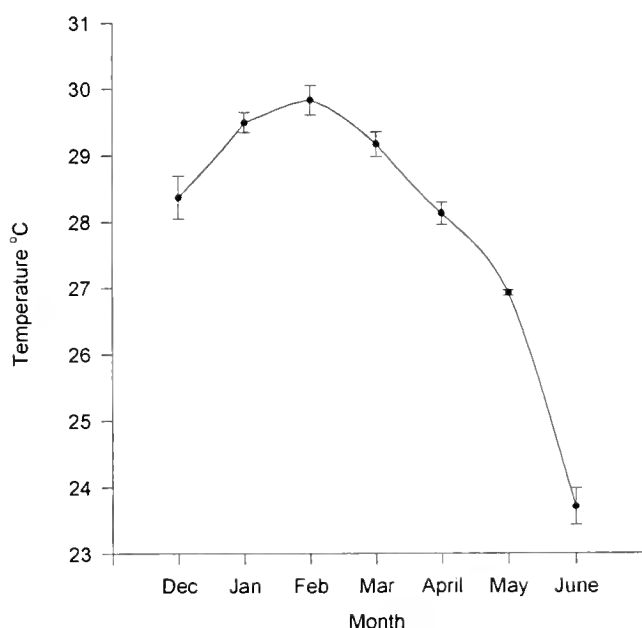


Figure 4. Changes in mean (\pm SE) monthly water temperature ($^\circ\text{C}$) at Pioneer Bay, Orpheus Island, during the nursery experiment.

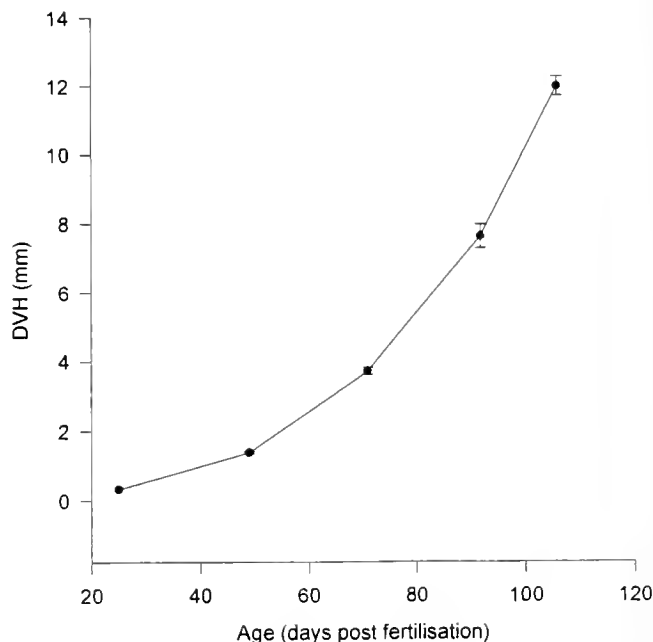


Figure 5. Changes in mean DVH of *P. margaritifera* spat during early nursery culture up to grading at 106 days old.

in spat with DVH greater than 3 mm. Spat growth was rapid, and 106-day-old spat had a mean DVH and HL of $11.2 \pm 2.1 \text{ mm}$ and $11.7 \pm 2.7 \text{ mm}$, respectively. The proportion of 106-day-old spat in each of four size categories, after removal from the settlement media and grading, is presented in Table 3. The majority of spat (67.3%) passed through the 10-mm-pore-size mesh and were retained on the 5-mm-pore-size mesh. Nine percent of the spat were retained on the 10-mm-pore-size mesh, and 0.2% were retained on the 15-mm-pore-size mesh. Almost 24% of the juveniles passed through the 5-mm-pore-size mesh. The largest individual measured at 106 days had a DVH of 23 mm, while the smallest was less than 2 mm. Survival of spat between transfer to the sea on Day 43 and grading on Day 106 was 38.9%. The relationships between DVH, APM, HL, and wet weight for *P. margaritifera* spat are described in Table 4.

Spat at all densities in both trays and pearl nets tended to aggregate and form clumps composed of many individuals. The number of spat in the clumps increased with increasing stocking density. Survival, wet weight (WW), and shell dimensions of spat held at different densities in trays are shown in Table 5. Survival of pearl oyster juveniles in plastic trays was high and varied be-

TABLE 3.

Percentage of *P. margaritifera* spat in each of four size classes when graded at 106 days of age.

Pore Size of Mesh (mm)*	Diagonal Measure (mm)	Juveniles Retained (%)
15	23	0.2
10	15	8.9
5	7	67.3
<5		23.6

* Three pore sizes were used for grading: 15, 10, and 5 mm, which had diagonal measurements of 23, 15, and 7 mm, respectively.

TABLE 4.

Morphometric relationships for *P. margaritifera* spat following log transformation of values for DVH, APM, HL and WW.

x	y	Regression Equation	r ²	n
DVH(mm)	APM (mm)	$y = 0.9944x - 0.0175$	0.985	1,067
DVH(mm)	HL (mm)	$y = 0.8543x - 0.386$	0.967	1,117
DVH(cm)	WW (g)	$y = 3.0294x - 9.288$	0.983	1,117

tween 76.6 and 88%. The majority of trays, including all replicates stocked with 10 oysters, two replicates stocked with 50 oysters, and one stocked with 100 oysters, became populated by leather-jackets (*Paramonacanthus japonicus*) during the study. These fish trimmed the dorsal shell margin and growth processes of spat shells and may also have ingested mantle tissue. The data presented in Table 5 include replicates affected by *P. japonicus*. DVH, APM, and WW were all higher at a density of 100 than at densities of 50 or 10 individuals per tray. Individuals held at a density of 100 per tray had significantly greater DVH, APM, HL, and WW than individuals held at a density of 50 per tray; however, there were no significant differences in DVH, APM, HL, or WW between oysters held at 100 per tray and those held at 10 per tray.

Survival, WW, and shell dimensions of spat held at different densities in pearl nets are shown in Table 6. Survival of individuals held in pearl nets was lower than that for pearl oysters held in trays and ranged from 68 to 74.8%. One of the pearl nets stocked with 20 oysters contained *P. japonicus*, and this replicate was not included in the data presented in Table 6. Pearl oysters held at a density of 20 per pearl net had greater DVH, APM, HL, and WW than those held at any of the other four densities; however, there were no significant differences for any of these parameters between oysters held at 20 per net and those held at 50 per net. There was a progressive decline in mean DVH, APM, HL, and WW with increasing stocking density, and spat held at densities of 20 and 50 per net had significantly greater DVH, APM, HL, and WW than those held at higher densities. The presence of *P. japonicus* significantly affected shell growth of juvenile oysters. For example, mean DVH, APM, HL, and WW of juveniles held at a density of 20 per pearl net were 36.77 (± 0.73) mm, 36.26 (± 0.68) mm, 32.96 (± 0.56) mm, and 5.96 (± 0.29) g, respectively, when the fish-affected replicate was included. However, when this replicate was omitted, mean values for DVH, APM, HL, and WW were 39.22 (± 0.65) mm, 38.36 (± 0.63) mm, 34.46 (± 0.54) mm, and 6.84 (± 0.80) g, respectively.

DISCUSSION

There is a paucity of information on successful spawning induction of *P. margaritifera*. In this study, cleaned broodstock were held overnight in a minimum volume of seawater in an air-conditioned room (ca. 22°C) before spawning induction. Spawning was readily induced the following morning, when broodstock were returned to ambient or heated (to a maximum of 32°C) seawater. At Orpheus Island, this method has been used successfully between September and May and has consistently resulted in the production of high-quality gametes. *P. margaritifera* at Orpheus Island experience an annual water temperature range of 20.1 to 31.2°C (B. Willis unpubl.); as such, the minimum temperature experienced by broodstock before spawning induction is within the range normally experienced in the wild. However, in regions where the natural temperature range of seawater is narrower than that experienced by the *P. margaritifera* used in this study, the minimum water temperature reached during "cold conditioning," before spawning induction, should be modified accordingly. It should also be noted that *P. margaritifera* broodstock often spawn spontaneously after transport to the hatchery or in response to cleaning.

The flow-through larval culture system was initially investigated as a means of simplifying hatchery procedure (Southgate 1995). Southgate and Ito (in press) suggested that a flow-through larval rearing system not only offered a simpler method of larval rearing but, because of more frequent water exchanges, would result in better water quality compared with that in conventional static culture systems. Although there were favorable and significant differences between water-quality parameters in the flow-through and static culture tanks, these did not promote significantly improved larval growth or survival. However, the flow-through tanks required only two complete water changes during the larval culture period compared with the five water changes performed on static-culture tanks. Clearly, one of the major potential benefits of a flow-through larval culture system is reduced labor input. This is likely to be particularly advantageous in small island nations of the Pacific, where the availability of skilled or experienced hatchery staff is extremely limited.

Large variation in the growth rates of juvenile *P. maxima* and *P. margaritifera* cohorts has been reported for both wild (Scoones 1990) and hatchery-cultured juveniles (Alagarwami et al. 1989, Rose 1990, Rose and Baker 1994). Similar variation in spat size was also recorded in this study. At 106 days of age, the largest individuals had a DVH greater than 20 mm, while the smallest had DVH measurements of less than 2 mm; approximately 9% of spat at this age were retained on a 10-mm-pore-size mesh, while 23.6%

TABLE 5.

Mean (\pm SE) survival, DVH, APM, HL and WW of *P. margaritifera* spat held at three densities in plastic trays for 19 weeks.

Density	Survival (%)	DVH (mm)	APM (mm)	HL (mm)	WW (g)
10	76.67 ^a \pm 3.33	37.39 ^{a,b} \pm 1.47 (18–45)	38.57 ^{a,b} \pm 1.64 (19–52)	35.70 ^{a,b} \pm 1.56 (17–45)	7.19 ^{a,b} \pm 0.61 (1.0–12.1)
50	88.00 ^a \pm 5.29	35.70 ^a \pm 0.66 (19–49)	35.32 ^a \pm 0.73 (19–52)	32.17 ^a \pm 0.67 (16–47)	5.46 ^a \pm 0.28 (0.8–13.4)
100	87.00 ^a \pm 1.15	40.48 ^b \pm 0.91 (16–60)	39.68 ^b \pm 0.93 (16–60)	35.44 ^b \pm 0.84 (14–48)	7.44 ^b \pm 0.43 (0.7–21.9)

Ranges are shown in parentheses. Means in columns with the same superscript are not significantly different ($p > 0.05$).

TABLE 6.

Mean (\pm SE) survival, DVH, APM, HL and WW of *P. margaritifera* spat held at five densities in pearl nets for 19 weeks.

Density	Survival (%)	DVH (mm)	APM (mm)	HL (mm)	WW (g)
20	73.75 ^a \pm 7.74	39.22 ^a \pm 0.65 (25–47)	38.36 ^a \pm 0.63 (26–46)	34.47 ^a \pm 0.54 (25–42)	6.84 ^a \pm 0.80 (2.2–11.2)
50	74.80 ^a \pm 2.42	37.30 ^a \pm 0.41 (24–49)	36.69 ^a \pm 0.43 (22–50)	32.66 ^a \pm 0.40 (18–47)	6.02 ^a \pm 0.18 (1.1–12)
100	70.40 ^a \pm 4.85	34.28 ^b \pm 0.58 (9–48)	32.50 ^b \pm 0.57 (10–46)	30.08 ^b \pm 0.56 (11–44)	4.40 ^b \pm 0.18 (0.1–10.6)
150	68.00 ^a \pm 2.89	30.63 ^c \pm 0.55 (10–53)	28.75 ^c \pm 0.54 (10–53)	26.59 ^c \pm 0.49 (11–43)	3.31 ^c \pm 0.17 (0.1–12.3)
200	68.30 ^a \pm 2.49	29.77 ^c \pm 0.58 (12–49)	28.40 ^c \pm 0.58 (13–51)	26.21 ^c \pm 0.50 (12–42)	3.24 ^c \pm 0.17 (0.1–11.3)

Means in columns with the same superscript are not significantly different ($p > 0.05$). Ranges are shown in parentheses. Means were calculated from five replicates per treatment, except at a density of 20 oysters per net, where data were calculated from four replicates.

fell through a 5-mm-pore-size mesh. To maintain the highest growth rates during nursery culture, regular grading is required to allow spat to be placed in growout apparatus with the largest suitable mesh pore sizes. The use of the crates and pearl nets in this study was related to the variation in size of the spat at first grading (106 days) as well as the desire to explore the value of different nursery culture techniques. The range of sizes at grading and the requirement for the largest mesh pore size to suit the juveniles led to the use of the two types of rearing systems. Continual grading during nursery culture ensures optimized growing conditions. At each grading, the pore size of the container is increased to match increasing juvenile size; this reduces fouling and ensures adequate water flow rates, which provide an adequate food supply and oxygen, and remove waste products (Gervis and Sims 1992). Achieving maximum growth rates in the nursery phase of pearl oyster culture reduces the time required to reach operable size for pearl production. Scoones (1990) reported that slower growing *P. maxima* juveniles in Western Australia required 30 mo to reach commercial size compared with 18 mo for the rapid growers. Smaller or slower growing pearl oyster juveniles require more frequent maintenance and have a longer nonproductive culture period.

It is interesting to note that at the end of the nursery trial, the largest pearl oysters held in pearl nets were of similar size and weight to those of the largest pearl oysters held in plastic trays. However, the pearl oysters stocked into plastic trays were those retained by a 10-mm-pore-size mesh during grading, whereas oysters used to stock pearl nets were those retained by a 5-mm-pore-size mesh. Although this may reflect differences in growth rates between oysters held in the trays and pearl nets, it is more likely to result from the effects of *P. japonicus*, which were far more common in trays than in pearl nets.

The *P. margaritifera* spat produced in this study showed growth rates similar to those reported for *P. margaritifera* in other studies. Alagarwami et al. (1989) reported a daily DVH growth rate of 0.4 mm/day for hatchery-reared *P. margaritifera* spat on transfer to the ocean; these animals had a mean DVH of 14.2 mm (range, 8.2–21.1 mm) 99 days after settlement. Growth data are also available for wild-collected *P. margaritifera* spat from French Polynesia. Coeroli et al. (1984) reported that spat held in suspended culture at 3 m reached a DVH of 8–10 mm after 3 mo and 40–50 mm after 6 mo. In the Solomon Islands, Friedman and Bell

(1996) reported that *P. margaritifera* spat removed from collectors that had been in the sea for 6 mo had a mean DVH of 32.4 ± 1.7 mm (range, 8–71 mm). The mean size of spat reported by Friedman and Bell (1996) is comparable to that in this study; when the nursery trial was terminated, spat were almost 7.5 mo old and had a mean DVH of approximately 40 mm. However, this is considerably smaller than the largest spat recorded by Friedman and Bell (1996), which had a DVH of 71 mm.

Survival of juvenile *P. margaritifera* between transfer to the sea and termination of the nursery trial ranged from 29.6 to 34.2% for juveniles held in trays and from 26.5 to 29.1% for juveniles held in pearl nets. This is relatively high compared with survival reported for *P. margaritifera* in India. Alagarwami et al. (1989) reared hatchery-produced *P. margaritifera* spat in pearl nets (triangular base with 35-cm sides) at a density of 600 per net. Forty-five days after transfer to the sea, survival was 15.1–17.4%; however, 50 days after transfer, survival had declined to almost zero (Alagarwami et al. 1989). This low survival may have resulted from the extremely high densities of spat in the pearl nets, although the authors stated that *P. margaritifera* does not occur naturally in the coastal waters of India, where their growth trials were conducted. In contrast, mortality of 6- to 12-mo-old *P. margaritifera* spat in French Polynesia has been reported at approximately 30% (Coeroli et al. 1984).

The gregarious behavior of *P. margaritifera* spat and their tendency to form clumps are consistent with the findings of previous studies. Crossland (1957) reported that *P. margaritifera* grown in mesh-covered boxes in the Red Sea readily formed "clusters," which if not broken-up, resulted in stunting or mortality of the innermost individuals. Similar behavior has been reported for spat of the Japanese pearl oysters, *Pinctada fucata* (Gervis and Sims 1992) and the silverlip pearl oyster *P. maxima* (Taylor et al. 1997). Taylor et al. (1997) reported that early juvenile *P. maxima* moved together to form large groups of up to 25 individuals when held at high stocking densities. This behavior resulted in reductions in shell growth, survival, and WW and an increase in the prevalence of growth deformities (Taylor et al. 1997).

Sims (1994) reported "fish grazing" as a cause of non-nacreous shell loss in *P. margaritifera* juveniles in the Cook Islands. Similar damage was caused to juveniles in this study by leatherjackets (*P. japonicus*; family *Aluteridae*). Groups of these fish took up residence in some of the trays and nets used in this

study and trimmed the non-nacreous shell margin, growth processes, and possibly some mantle tissue from juvenile oysters. The actions of these fish caused significant reduction in juvenile growth. The trays and nets used in this study were brushed on the outside to remove fouling but not inspected internally during the study. The presence of *P. japonicus* could have been prevented by regular and thorough inspection of culture apparatus. Many of the fish found at the end of the nursery trial were too large to escape through the mesh of the trays and nets and were trapped within them. Fish, primarily of the family *Balistidae*, have been recorded as predators of juvenile *P. margaritifera* in the Solomon Islands (Friedman and Bell 1996) and as a source of mortality of *P. margaritifera* in the Red Sea (Crossland 1957). Other recorded predators of *P. margaritifera* spat and juveniles include crabs and gastropods such as *Murex* spp. and *Cymatium* spp. (Crossland 1957, Southgate and Beer 1996, Friedman and Bell 1996). Although

predation by *Cymatium* is a major problem for bivalve culture in other parts of the Pacific (Govan 1995, Friedman and Bell 1996), they are rarely encountered on suspended culture apparatus used at Orpheus Island.

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EFFECTS OF STOCKING DENSITY ON THE GROWTH AND SURVIVAL OF JUVENILE SILVER-LIP PEARL OYSTERS (*PINCTADA MAXIMA*, JAMESON) IN SUSPENDED AND BOTTOM CULTURE

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ABSTRACT Growth and survival of juvenile silver-lip (or gold-lip) pearl oysters, *Pinctada maxima*, were compared at two stocking densities (28 individuals per net: 66 oysters per m² or 48 individuals per net: 99 oysters per m²) with animals held in either suspended or bottom culture. The experiment was terminated during the sixth week because of high mortality in bottom-cultured pearl oysters. Mean (\pm SE) survival in 28-pocket nets in suspended culture ($99.0 \pm 1.6\%$) was significantly better than that in any other treatment ($p < 0.01$). Survival was also high in the 48-pocket nets in suspended culture ($94.8 \pm 3.6\%$). Mean survival in bottom culture was significantly lower ($p < 0.05$), being 15.8 ± 7.8 and $13.3 \pm 3.6\%$, respectively, for 28 and 48-pocket nets. *P. maxima* held in suspended culture grew significantly larger ($p < 0.001$) than those in bottom culture. In both suspended and bottom culture, *P. maxima* in the 28-pocket nets grew larger ($p < 0.001$) than those held in 48-pocket nets. Additionally, pearl oysters held in bottom culture had brittle shell margins. These results indicate that culture system had a greater influence on growth and survival than stocking density. Differences in the availability of food are believed to be the major influence on the results obtained; the dry weight of suspended solids, phytoplankton biomass, and phytoplankton diversity were all greater in surface waters.

KEY WORDS: pearl oyster, *Pinctada maxima*, suspended culture, bottom culture, growth, stocking density, pocket nets

INTRODUCTION

Recent years have seen rapid developments in the hatchery production of silver-lip (or gold-lip) pearl oyster (*Pinctada maxima*) seed in Australia and southeast Asia (Gervis and Sims 1992, O'Sullivan 1994, Rose 1994). However, there is a paucity of published information concerning growout techniques for this species. Stocking density is a major factor affecting survival, growth, and the level of growth deformity of *P. maxima* spat during the nursery phase (Taylor et al. 1997); however, nursery culture is only the first stage of growout and there is scant information available on the effects of stocking density on larger juvenile and adult pearl oysters.

The effects of stocking density on growth and survival are well documented for bivalves such as clams (Hadley and Manzi 1983, Hurley and Walker 1994), edible oysters (Jaryaband and Newkirk 1989, Arakawa 1990, Roland and Albrecht 1990, Holliday et al. 1991, Holliday et al. 1993, Rheault and Rice 1996), and scallops (Duggan 1973, Parsons and Dadswell 1992, Gaudet 1994). The type of system used for commercial bivalve culture also influences growth, survival, and the cost of production (Duggan 1973, Spencer and Gough 1978, Toro and Varela 1988, Gayton-Mondragon et al. 1993). In particular, the position of a bivalve within the water column influences factors such as food availability, seston levels, rate of water exchange, temperature, salinity, and the level of fouling (Leighton 1979, Wilson 1987, Brown and Hartwick 1988a, Brown and Hartwick 1988b, Cote et al. 1993, Smitasiri et al. 1994).

Pearl oyster farmers often use bottom culture systems to hold adult pearl oysters. These may be simple "shell dumps," where newly fished animals are placed in a particular spot on the sea floor before entering the farm, or "bottom-line" systems where pearl

oysters are held in pocket (or panel) nets tied to a rope anchored along the sea floor. Pearl farmers often use a bottom-line system to hold pearl oysters during the "pearl-turning" program adopted after pearl operation (Gervis and Sims 1992). In this system, nets holding newly operated pearl oysters are regularly turned such that the side previously face down is face up. Most operators believe that this encourages better development of the pearl sac—the newly grafted mantle tissue within the oyster gonad that is responsible for nacre deposition over the pearl nucleus (Scoones 1990, Gervis and Sims 1992). One advantage of this system is that it reduces the need for regular cleaning because the turning process itself helps reduce fouling. Additionally, anecdotal evidence suggests that survival of postoperative pearl oysters is higher when placed on the sea floor than in suspended culture near the sea surface. The aim of this study was to assess the effects of both stocking density and culture method (suspended or bottom culture) on the growth and survival of juvenile *P. maxima*.

MATERIALS AND METHODS

P. maxima spat were hatchery propagated following the general methods described by Rose (1990). Spat that had settled onto collectors were placed in suspended culture at sea once they had reached an anteroposterior shell length of approximately 3 mm (at between 35 and 40 days of age). Juvenile *P. maxima* were removed from collectors after 10 wk at sea by severing the byssal attachment with a scalpel blade; they were then graded by size. Individuals with mean (\pm SE) shell height and hinge length of 30.5 ± 2.1 and 34.1 ± 1.9 mm, respectively, were selected for the study. Individuals were either stocked into nets (frame size, 500×850 mm; Fig. 1) with 48 pockets (pocket dimensions, 8×10 cm; 99 oysters per m²) or 28 pockets (pocket dimensions, 12×12 cm; 66 oysters per m²) (Fig. 1). Seven of each net type were suspended at

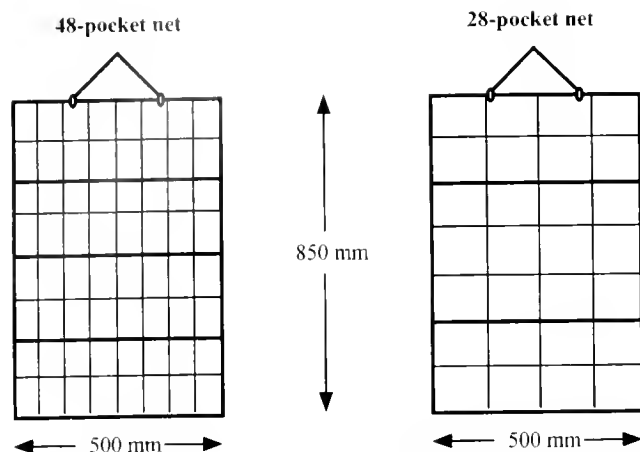


Figure 1. Forty-eight-pocket and 28-pocket nets used to house juvenile *P. maxima*.

1-m intervals from a surface longline to a depth of 2.5 m (suspended culture), and a further seven of each net type were placed flat on the sea floor (coarse sand) at a depth of 20 m (bottom culture). Nets held on the surface were cleaned with a high-pressure seawater jet approximately every 10 days. Nets on the sea floor were turned weekly to minimize fouling.

Water temperatures near the sea surface and on the sea floor were recorded weekly. Water samples were taken every 2 wk, and 2 L of water from each site was filtered with a preweighed Whatman GF/C filter fitted to a vacuum flask. The filter was then dried for 24 h at 40°C and reweighed to determine the dry weight of suspended solids. To measure food availability, two 1-L samples of seawater from each site were filtered with the above equipment. Phytoplankton collected on the GF/C filters was resuspended in 1 mL of seawater (previously filtered to 1 µm), and the numbers of morphologically different phytoplankters were counted microscopically with a Sedgwick-rafter counting cell.

During the fifth week of the experiment, heavy mortality became evident in pearl oysters held in bottom culture. During the sixth week, the trial was terminated and the numbers of surviving animals from each system were counted. Dorsoventral shell height and hinge length measurements were taken for all of the surviving individuals held in bottom culture, and the same measurements were taken for 20 randomly selected animals from each net in suspended culture.

Shell height and hinge length data were compared by one-way analysis of variance (Sokal and Rohlf 1981), and means were compared using Fisher's Protected Least Significant Difference test, from the Statview computer program for Macintosh computers, version 4.02 (Abacus Concepts, StatView 1992). Percent survival data were arcsin transformed before analysis (Sokal and Rohlf 1981). Homogeneity of variances was confirmed using Cochran's test (Snedcore and Cochran 1967).

RESULTS

Survival was affected by both stocking density and culture system. Large numbers of dead pearl oysters were observed in the bottom culture system at the end of the fifth week, and the trial was terminated during the sixth week. No evidence of attack on the juvenile pearl oysters by predatory animals was observed in either surface or bottom culture systems. Survival of pearl oysters at the end of the experiment is shown in Figure 2. Mean (\pm SE) survival of oysters held in 28-pocket nets in suspended culture was 99.0 \pm

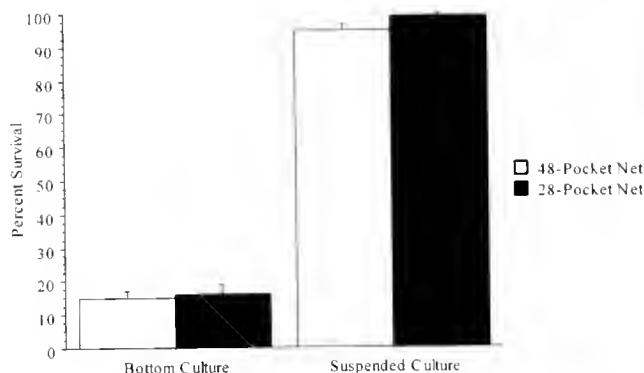


Figure 2. Percent survival (mean \pm SE; $n = 7$) for juvenile *P. maxima* cultured for 6 wk in either suspended culture or bottom culture in pocket nets holding either 48 or 28 individuals.

1.6% and was significantly higher than in any other treatment ($p < 0.05$). Survival was also high in the 48-pocket nets in suspended culture (94.8 \pm 3.6%). Mean survival of pearl oysters held in bottom culture was significantly lower than in either stocking density in suspended culture ($p < 0.05$), being 15.8 \pm 7.8 and 13.3 \pm 3.6% for 28- and 48-pocket nets, respectively. These values did not differ significantly ($p > 0.05$; Fig. 2).

Shell growth (shell height and hinge length) was also affected by culture system (Table 1). Juvenile *P. maxima* held in suspended culture were significantly larger ($p < 0.001$) than those in bottom culture, and those held in the 28-pocket nets were significantly larger ($p < 0.001$) than those in the 48-pocket nets. Additionally, pearl oysters held in bottom culture had noticeably thinner shells and brittle shell margins compared with those held in suspended culture.

The total phytoplankton count per liter of seawater (Fig. 3) and the diversity of phytoplankton species (Table 2) were always greater in surface waters than in water adjacent to the bottom. Additionally, the dry weight of suspended solids was always higher in surface water samples (Table 2). The mean water temperature 2.5 m from the surface over the 6-wk period was 29.5 \pm 0.2°C (\pm SE); the mean water temperature on the sea floor was 28.8 \pm 0.1°C (\pm SE).

DISCUSSION

Both the density and the type of culture system affected the growth and survival of juvenile *P. maxima*. However, differences in growth and survival were influenced more by culture system than stocking density. Differences in survival between the stocking densities tested within each culture type were not great, and for pearl oysters cultured on the sea floor, the difference was not

TABLE 1.

The mean (\pm SE; $n = 7$) shell heights and hinge lengths for juvenile *P. maxima* cultured for 6 wk in either suspended (SC) or bottom culture (BC) in pocket nets holding either 48 or 28 individuals.

No. of Pockets per Net	Shell Height		Hinge Length	
	SC	BC	SC	BC
48	43.9 \pm 0.3 ^a	34.9 \pm 0.8 ^b	43.8 \pm 0.4 ^a	39.1 \pm 1.0 ^b
28	48.1 \pm 0.3 ^c	38.0 \pm 0.7 ^d	49.5 \pm 0.4 ^c	42.6 \pm 0.9 ^d

Values for shell height or hinge length (HL) with different superscripts are significantly different ($p < 0.001$).

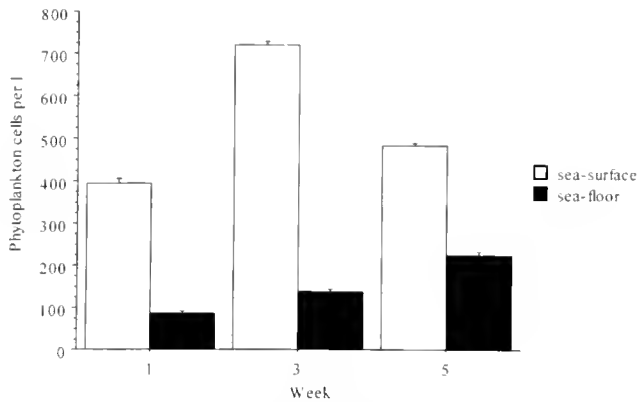


Figure 3. The number of phytoplankton cells (mean \pm SE; $n = 2$) per liter of seawater sampled 2.5 m below the sea surface and at a depth of 20 m on the sea floor.

significant. Best survival and growth were shown by juveniles held in 28-pocket nets (66 oysters per m^2) suspended from a surface longline. The mean shell heights and hinge lengths of *P. maxima* held in suspended culture were greater than those in bottom culture, regardless of stocking density. Juvenile pearl oysters held in 28-pocket nets in suspended culture were, on average, approximately 5 mm longer along the height and length axes than those in 48-pocket nets (99 oysters per m^2) in suspended culture. However, the same animals were almost 10 mm longer along the height and length axes than those in 28-pocket nets held on the sea floor. The effect of culture system on growth was therefore far greater than that of density.

A major difference between surface seawater and that near the sea floor was the amount of food available to juvenile *P. maxima*. Water samples from near the surface always had higher phytoplankton counts, diversity of species, and level of suspended solids than did samples taken from the sea floor. The results suggest that growth and survival of *P. maxima* held on the sea floor were influenced by reduced food availability. Similar results have been shown in a number of growth studies with bivalves (Brown and Hartwick 1988b, Leighton 1979, MacDonald 1986, Numaguchi 1994). Numaguchi (1994) attributed slower than normal growth of the pearl oyster *Pinctada fucata martensii*, in Ohmura Bay, Japan, to low food abundance. Similarly, slower growth rates of giant scallops, *Placopecten magellanicus*, cultured on the bottom, compared with those in suspended culture, reflected lower food levels between sites (MacDonald 1986). Rock scallops, *Hininites multirugosus*, showed suppressed growth at depths equal to or greater than 60 m compared with scallops at shallower depths (30 m or less); the biomass of phytoplankton was much less at depths greater than 50 m (Leighton 1979). The same study showed

TABLE 2.

The dry weight of suspended solids (DWSS) and the number of morphologically different phytoplankters (NMDP) counted from water samples taken 2.5 m from the sea surface (SS) and at a depth of 20 m on the sea floor (SF).

Week	DWSS ($g \cdot 2 L^{-1}$)		NMDP (L^{-1})	
	SS	SF	SS	SF
1	0.02	0.01	57	44
3	0.02	0.01	69	53
5	0.02	0.01	87	58

that scallops held at the greater depths had thin fragile shells; this was also the case in this study, where *P. maxima* held in bottom culture developed brittle shell margins and thin shells. Wilson (1987) suggested that low food availability and the reduced growth rates of *Ostrea edulis* and *Pecten maximus* that resulted were worse where tidal currents were low. Wilson (1987) suggested that low tidal flow does not allow renewal of the food resources depleted by bivalves as they feed. This may have influenced the results in this study because currents were reduced near the sea floor.

The results strongly indicate that a major factor influencing the growth of pearl oysters in this study was food availability. However, other factors such as disease and/or disturbance from fish and benthic animals may also have influenced the results. At the time of this study, commercial trials of bottom culture were attempted in other sites with similar results. Bottom culture was clearly not suitable for juvenile *P. maxima* at the site used in this study, even though it is widely used for adult silver-lip pearl oysters (Gervis and Sims 1992).

Culture system and stocking density are major factors influencing the economics of bivalve aquaculture (Askew 1978, Roland and Albrecht 1990, Holliday et al. 1991, Parsons and Dadswell 1992, Holliday et al. 1993). On the basis of the results of this study, suspended culture at the lower stocking density in 28-pocket nets (66 oysters per m^2) is appropriate for juvenile *P. maxima*. Furthermore, the pockets of these nets are sufficiently large to house oysters up to 100 mm in size (J. J. Taylor unpubl.); their use could minimize the frequency of net changes during growout and reduce operational costs.

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LARGE-SCALE ANESTHESIA OF THE SILVER-LIP PEARL OYSTER, *PINCTADA MAXIMA* JAMESON

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ABSTRACT Propylene phenoxetol was evaluated as an anesthetic both in the laboratory and field during gonad conditioning trials of *Pinctada maxima*. It was found to be safe and effective for adult pearl oysters, inducing rapid anesthesia with short recovery times. Suitable concentrations were 1.5 mL L⁻¹ in the laboratory and 2–2.5 mL L⁻¹ in the field. Prior cleaning of oysters and minimising stress are critical to the effectiveness of the anesthesia protocol.

KEY WORDS: pearl oyster, *Pinctada maxima*, anesthetic, bivalve broodstock

As part of a program to develop protocols for the gonad conditioning of the silver-lip pearl oyster, *Pinctada maxima*, there was a necessity to be able to examine the reproductive condition of broodstock and to biopsy the gonads, without imposing excessive stress. Pearl oysters, especially those that have been hatchery reared, have large and powerful adductor muscles, and it is quite common to cause severe muscle and mantle damage when they are forced open for examination. This is not appropriate when the object is to induce the oysters to reproduce under captive conditions, which commonly means maintaining the broodstock under ideal conditions of temperature, food, and water quality in a quiet, low-stress environment.

Magnesium chloride has been successfully used to anesthetize broodstock scallops and was useful in preventing unwanted spawnings subsequent to physical examination (Heasman et al. 1995); however, this method produced poor results with pearl oysters, requiring 1–2 h to induce anesthesia and a similar time to recover (Mills unpubl.). Norton et al. (1996) screened many potential anesthetics for *Pinctada margaritifera* and *Pinctada albina* and concluded that, in particular, propylene phenoxetol (1-phenoxy-propan-2-ol) was very effective. Previously, this anesthetic had also been used successfully for giant clams (*C. Shelley* pers. comm.) and *P. margaritifera* (Hildemann et al. 1974).

The use of propylene phenoxetol for inducing anesthesia in the silver-lip pearl oyster, *P. maxima*, has since been applied on a large scale and has proved successful. During the course of this program, several thousand oysters have been anesthetized, with negligible mortality. Some of these oysters were anesthetized weekly for 9 wk without any evident ill effects. In broodstock conditioning trials, there have been no deleterious effects of anesthesia and gonad biopsies on either the growth or the gonad development of broodstock pearl oysters ($p > 0.05$). Currently, several pearl farm-

ing companies are trying the use of propylene phenoxetol in seedling operations.

Propylene phenoxetol has several advantages over other anesthetics. According to the manufacturer, as a 1% solution, it is nontoxic and nonirritating and does not induce skin hypersensitivity. Anesthesia of *P. maxima* is rapid and safe, with a short recovery period. There are no special storage or safety procedures required, and it is not an explosion hazard. However, it should not be stored in some plastics, notably polycarbonate.

The anesthetic may be used at concentrations between 1 and 3 mL L⁻¹. Concentrations of 2–3 mL L⁻¹ will induce a rapid and relatively deep anesthesia, with a subsequently longer recovery period (Norton et al. 1996). In this study on *P. maxima* from 120 to 2,000 g, the optimal concentration for use in the laboratory has been found to be 1.5 mL L⁻¹ in the laboratory and 2–2.5 mL L⁻¹ in the field.

The anesthetic should be added at the appropriate concentration, and the solution should be aerated. Once the anesthetic is dissolved, aeration is not required, except to maintain dissolved oxygen levels, and may otherwise make observing the oysters difficult. Oysters to be anesthetized should be gently placed hinge down in the solution, leaning against the edge of the tank. This position allows the oyster to be easily monitored. Anesthesia generally takes from 6 to 15 min at temperatures between 24 and 32°C, at which time the oyster will be gaping and unresponsive to handling. At temperatures below 24°C, anesthesia time is increased. A well-anesthetized oyster will gape sufficiently wide to part the gill curtain inside the shell. After examination, the oyster should be placed into clean, aerated or flowing seawater.

There are two main factors that control the success of the anesthesia: these are the degree of stress that the oyster is subjected to immediately before being anesthetized and the degree of biofouling on the shell. If the oyster is stressed before being placed

into the bath, it will remain closed for some time, and therefore, anesthesia will be delayed. In addition, when the oyster does open and becomes anesthetized, the shell will be only slightly open (2–4 mm) and will still require the use of shell-opening forceps to open the shell sufficiently for examination. Anesthetized oysters with only slightly open shells may be easily overlooked, resulting in prolonged exposure to the anesthetic and hence a very deep anesthesia and a long recovery time. This may further result in mantle collapse or in an oyster vulnerable to predators if placed straight back into the sea. As a general rule, the degree of gaping is inversely proportional to the stress level of the oyster when placed into the bath. Stress may be produced by transport, such as on a boat in choppy seas, by rough handling, or by cleaning.

Cleaning the shell before anesthesia is essential, because otherwise, the chemical is absorbed by the biofouling and mud, resulting in a rapid decline in concentration. Generally, it will be

difficult to remove all of the fouling from an oyster on the farm, and therefore, a higher anesthetic concentration of 2–2.5 mL L⁻¹ is recommended. Once the anesthesia time for the oysters increases to 20–30 min, the solution should be replaced. Because preanesthesia stress must be avoided, the oysters should be cleaned the day before being anesthetized.

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ERRATUM

The author's name and address for the "In Memoriam" for R. Tucker Abbott that appeared in Volume 15(2) pp. 185-190 was omitted. Authorship should be attributed to Dr. Melbourne R. Carrier, College of Marine Studies, University of Delaware, Lewes, Delaware 19958-1298.

The printer regrets the error.

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COVER PHOTO: *Homalaspis plana* "jaiba mora" at Caleta El Quisco artisanal cove, central Chile (photograph by Antonio Larrea)

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